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BIOLOGICAL REVIEWS

of the

Cambridge Philosophical Society



Edited
by
H. MUNRO FOX

VOLUME 14

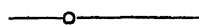
CAMBRIDGE
AT THE UNIVERSITY PRESS
1939

PRINTED IN GREAT BRITAIN

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THE EARLY EVOLUTION AND RELATIONSHIPS OF THE ELASMOBRANCHS

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(Received 29 January 1938)

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I. INTRODUCTION

FOR the purpose of this article, the term "Elasmobranchii" will be used synonymously with the term "Chondrichthyes", that is, including only the cartilaginous shark-like fishes. In the past authors have used the term in rather different senses, and it has been used to include a number of early groups of fishes, which in reality show no very close relationship with the Chondrichthyes proper. It would be valueless and outside the scope of this paper to provide a review of the uses of the term elasmobranch, since the various writers have not always intended them to correspond. Here we are only concerned with the early evolution of those cartilaginous fishes, distinguished from all other gnathostomes by the possession of a skeleton of cartilage, which if calcified forms only a superficial layer of prismatic granules, but never true bone. There are, however, a number of early "shark-like" fishes, termed here the Placodermi, including the Arthrodira, Antiarchi, Macropetalichthyda, Rhenanida and Acanthodii, which have been assigned various degrees of affinity with the Chondrichthyes by most recent authors, among whom may be mentioned Säve-Söderbergh (1934), Romer (1933, 1937), Broili (1930 *a*, 1933 *a*, *b*), Stensiö (1934 *a*, *b*, 1936), White (1936, 1937), Gross (1937 *a*, *b*). Only Watson (1937)

and Westoll (1937) have denied that any close affinity exists between the Chondrichthyes and the Placodermi, believing the latter, for which Watson suggests the name *Aphetohyoidea*, to be at an intermediate grade of organization between that of the Agnatha and that of the later Gnathostomata.

The elasmobranchs, apart from various spines and a few teeth of uncertain affinities, first occur in the Upper Devonian, where they are represented by exceedingly primitive types. The Placodermi on the other hand are already highly specialized during the Lower and Middle Devonian. This is important, for it is by far the strongest evidence that the Chondrichthyes are a distinct group, and that superficial resemblances between certain of the placoderms and the more highly developed elasmobranchs are not signs of close affinity. The possibility that the elasmobranchs were derived from some placoderm-like form is not denied, it is only emphasized, that the placoderm groups, as at present known, are far more closely related to one another, and probably in a lower grade of evolution than the elasmobranchs. It is hoped that the following account of the evolution of the Chondrichthyes and comparison with the various placoderms will make it clear that there is no reason for considering that they are closely related or should be classified together.

II. THE EVOLUTION OF THE ELASMOBRANCHS

The evolution and classification of the elasmobranchs has already been fully treated by many authors, among whom may be mentioned Regan (1906, 1929), Goodrich (1909, 1930), Smith Woodward (1921), Norman (1931), Romer (1933) and White (1936, 1937), but recent research on Palaeozoic elasmobranchs necessitates a modification of their views (Moy-Thomas, 1936 *d*). Since the skeleton is usually the only part fossilized, the evolution of the Vertebrata is largely a history of this, but in the elasmobranchs there is the additional difficulty, that the skeleton owing to its cartilaginous nature is seldom well preserved and if it is, the state of preservation often makes interpretation very difficult. Teeth, head spines or fin spines form the large majority of the fossil remains of elasmobranchs; in some cases whole groups being defined by these alone. It is therefore possible that too much emphasis is laid on relatively unimportant characters such as the microscopic structure of the teeth (Carroll, 1929; Nielsen, 1932). On the other hand, it is in many cases the only means available of showing affinity, and although unsatisfactory must be used. The present knowledge of fossil elasmobranchs is so limited, that the following classification should only be considered as provisional.

Throughout the group from the earliest Upper Devonian forms two types of tooth structure are recognizable as Nielsen (1932) has shown. It is proposed to call the two groups characterized by these teeth types the *Selachii* and *Bradyodonti*. Since all elasmobranchs apparently fall into one or other of these groups, they may be assumed to constitute the two major lines of elasmobranch evolution. The microscopic structure (Fig. 1 A) of a selachian tooth consists typically of an inner core of osteodentine, surrounded by a layer of orthodentine, which is covered

superficially by a layer of "enamel". The bradyodont tooth (Fig. 1 B) has an inner layer of highly vascular dentine surrounded by a crown of vertical parallel dentine tubules, but with no outer layer of "enamel". The term bradyodont was invented by Smith Woodward (1921) to include a number of groups whose teeth have this microscopic structure with slow replacement, but Nielsen has extended its use to include such forms as the edestids and orodonts with more rapid tooth succession, and here it is further extended to include the Holocephali. Nielsen appreciated this rather illogical use of the term, but found it convenient to use it in this sense.

The existence of two distinct evolutionary lines in the elasmobranchs raises another question of great interest. In all the bradyodonts, in which it is known, the jaw suspension is of the holostylic type and there is every possibility that this is a characteristic of the group as a whole. De Beer & Moy-Thomas (1935) have pointed

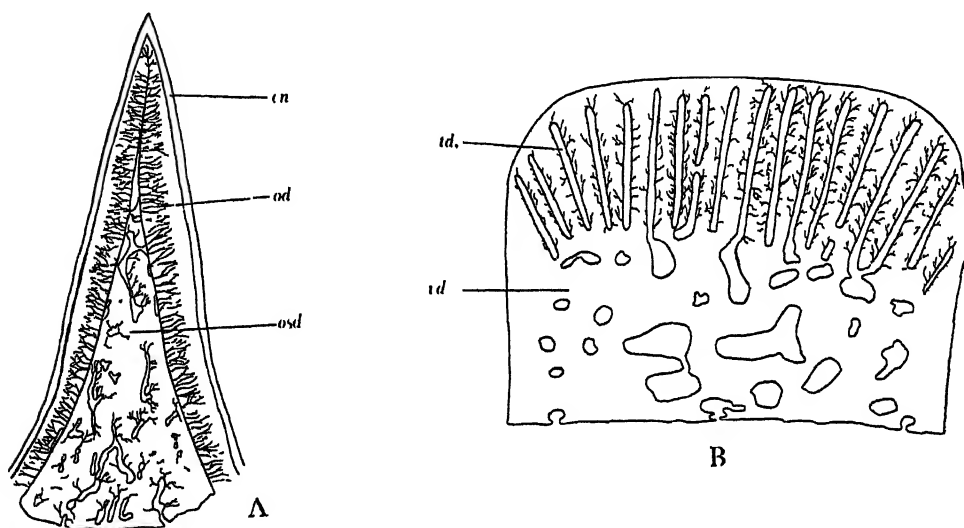


Fig. 1. A. Section of selachian tooth. *en.* enamel, *od.* orthodentine, *osd.* osteodentine.
B. Section of bradyodont tooth. *td.* tubular dentine, *vd.* vascular dentine.

out that "chief among the primitive features of the Holocephali is the presence of a pharyngohyal element in the hyoid arch, which seems to point to the conclusion that their ancestors never were amphistylic or hyostylic". There is every reason to suppose that the Elasmobranchii are descended from ancestors in which the hyomandibular was not suspensorial, the amphistylic and hyostylic conditions in the Selachii being independently evolved from those in other fishes (Allis, 1918). The Cladoselachii, probably the most primitive selachian type, are however, amphistylic, so the holostylic bradyodonts must, on such a view, have originated in a pre-cladoselachian stage of evolution. On this view it must be assumed that the pelvic claspers found in both selachians and bradyodonts have been independently evolved, since they are not found in the Cladoselachii. But the rather specialized nature of the claspers, the fact that in all known bradyodonts the

holostyly is associated with a somewhat crushing dentition, together with Watson's (1937) new interpretation of the elements of the hyoid arch in the living Holocephali, suggests that it is far more probable that the non-suspensory condition is secondary.

The evolution of the Selachii and Bradyodonti will be treated separately below. It should, however, be emphasized that no one has denied that they are closely related and together form a natural group, the Elasmobranchii.

(a) *Selachii*

The earliest known selachians, the cladodonts occur in the Upper Devonian.¹ They have the characteristic "Cladodus" tooth consisting of a large central cusp with usually two or more small lateral cusps on either side. The cladodonts, which are the only selachian type found in the Devonian, continue into the Carboniferous, where they probably represent numerous families. However only two types of cladodonts can be readily distinguished owing to the very fragmentary nature of the remains. These are the Cladoselachii and the Ctenacanthii.

The known cladoselachians (Fig. 2 A) (Dean, 1894, 1909), although not entirely unspecialized, must have approached very closely the ancestral elasmobranch type of structure. *Cladoselache* the best known, has the body covered by a shagreen of typical placoid denticles (Woodward & White, 1938), paired fins which are triangular lateral folds of the body, a strongly heterocercal tail and two dorsal fins, in all of which fins the unjointed cartilaginous radials extend to the margin of the fin. The dorsal fins are usually without anterior fin spines, but in some species (Harris, 1938 a), possibly only in the males there is a small laterally compressed spine in front of the anterior dorsal fin. This spine is a superficial structure and has no large area inserted into the body between the myotomes as in ctenacanth and hybodonts. Along the proximal margin of the uniserial paired fins is a row of basal cartilages which vary in number and arrangement in different forms (*Cladoselache*, Dean, 1894, 1909; *Symmorium*, Cope, 1893, 1894; *Danaea*, Pruvost, 1922; Fournier & Pruvost, 1928; "*Cladodus*" *neilsoni*, Traquair, 1897), but show some kind of concentration anteriorly in connexion with the formation of pectoral and pelvic girdles. The pelvic fins are of particular interest as there is apparently no development of "claspers", which are so characteristic of other elasmobranchs. The neurocranium, well known in "*Cladodus*" *wildungensis* and *Cl. hassiacus* (Holmgren & Stensiö, 1936; Stensiö, 1937; Gross, 1937 a, 1938) and *Cladoselache* (Harris, 1938 b) is very similar to that of the modern *Chlamydoselachus*. The jaw suspension is amphistylic. There is no development of centra in the vertebral column, and no anal fin; the suggestion that it is represented by the paired lateral flaps just anterior to the caudal fin is not very convincing.

The cladoselachian type of paired fin has long been used as evidence of the fin-fold theory of the origin of paired fins, but recently this has been challenged

¹ The Middle Devonian and earlier remains sometimes attributed to the elasmobranchs which have been summarized by Romer & Grove (1935) and Watson (1937) are of too uncertain affinity to be considered.

by some writers, (Gregory, 1936; White, 1936, 1937) who maintain that the view is untenable since the geologically older acanthodians and macropetalichthyds (see below) have definitely concentrated fin skeletons. From a study of the elasmobranchs themselves there appears to be no doubt that the cladoselachian fin is

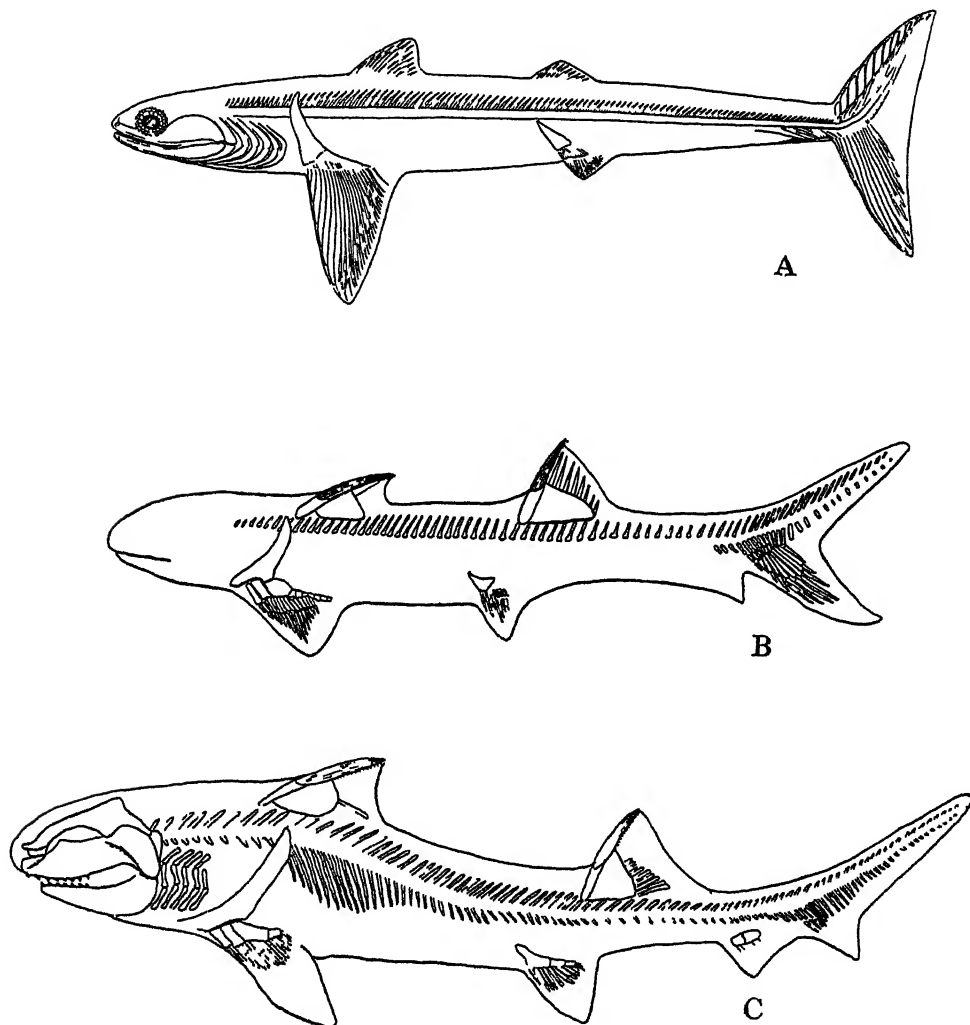


Fig. 2. A. *Cladoselache fylersi*, from Dean (1909). B. *Ctenacanthus costellatus*, from Moy-Thomas (1936b). C. *Hybodus hauffianus*. Restorations in lateral view.

primitive for the group, and its late appearance in this primitive condition may be explained on the assumption that these forms have evolved from an unknown ancestral group which retained the fin-fold. A further possible explanation is that the elasmobranchs are derived paedomorphically from bony ancestors retaining the larval characters of cartilage and fin-folds. Whichever of these views is correct

it does not alter the fact that the fin-fold is certainly primitive in the elasmobranchs, and probably in all fishes.

The teeth of the Devonian *Cladoselache* are interesting in not having developed the outer layer of enamel (Dean, 1909; Claypole, 1895), thus approaching the bradyodonts. This, however, may not be true of the Carboniferous forms included in the group, but at present there is no evidence on this point.

The ctenacanth (Fig. 2 B) are a group which has only recently been described in detail: their anatomy is known from a single Devonian specimen *C. clarki* (Dean, 1909) and from three Carboniferous specimens two of *C. costellatus* (Traquair, 1884; Smith Woodward, 1921; Brough, 1935; Moy-Thomas, 1936b), and one of *Goodrichia* (Moy-Thomas, 1936b). The ctenacanth differs from the *Cladoselachii* most obviously in having two dorsal fins with characteristically ornamented fin spines, the anterior one of which always makes a smaller angle with the long axis of the body than the posterior, and never has more than one radial above the triangular basal cartilage, while the posterior dorsal fin has several. The tail is strongly heterocercal and the anal fin is always situated very close to it. The fins show a general advance towards the modern type of elasmobranch fin in having the cartilaginous radials of the paired and caudal fins jointed at least once and not extending so near the margin of the fin as they do in the *Cladoselachii*. The pectoral fins of *C. costellatus* are particularly interesting. Although they are still triangular fin-folds, the anterior basals have become concentrated into three elements connected with the pectoral girdle, the pro-, meso-, and metapterygia. There is no evidence that this group had claspers, the jaw suspension is amphistylic and there are no centra in the vertebral column.

The ctenacanth has been considered to be closely related to the *Cladoselachii* by Smith Woodward (1921, 1932), but Brough (1935) considered them to be hybodonts. The Hybodontii (Fig. 2 C) are a group ranging from the Permian (probably upper Carboniferous) to the Cretaceous, which resemble the ctenacanth, as Brough pointed out, in the angles of the dorsal fin spines and internal skeleton of the dorsal fins, the position of the anal fin, and the tribasal pectoral fin. The hybodonts, however, have the radials of the paired fins jointed more frequently than the ctenacanth, and the axis of the basals in the pectoral fin is very much reduced, the fin no longer being a triangular fin-fold. The hybodonts also differ in the structure of the caudal fin which has the radials almost entirely reduced. The two halves of the pelvic girdle are separate and the basipterygium segmented, both primitive characters, but pelvic claspers are present in the male. The neurocranium is very similar to the modern *Chlamydoselachus* (Smith Woodward, 1916a), the jaws are amphistylic, the teeth show considerable variation in pattern, and there are no vertebral centra.

Despite these differences there does seem to be no doubt that the hybodonts are descended from the ctenacanth, and the latter from the *cladoselachians*. The course of this evolution may be summarized as being a jointing of the radials and withdrawal from the edge of the fin in both paired and unpaired fins, and the formation of the lobed elasmobranch tribasal fin from the fin-fold of *Cladoselache*.

In the Carboniferous and Permian there must have been many families of sharks distinct from the hybodonts and cladodonts, but obviously derived from the latter. Of these the most easily recognizable are the Pleuracanthode, but other groups must have existed and in at least two cases can be distinguished.¹ Brough (1935) mentions a shark of this kind, and *Petrodus* (Moy-Thomas, 1935 *b*) may be distinct from the hybodonts. *Tristychius arcuatus* (Fig. 3 A) (Traquair, 1888 *a*; Smith Woodward, 1924 *a*; Moy-Thomas, 1936 *b*) clearly represents a distinct type. In *Tristychius* the two dorsal fin spines make the same angle with the long axis of the body, the anal fin is well anterior from the caudal, the dibasal pectoral fin has radials once jointed extending to its margin, the caudal fin has no separate

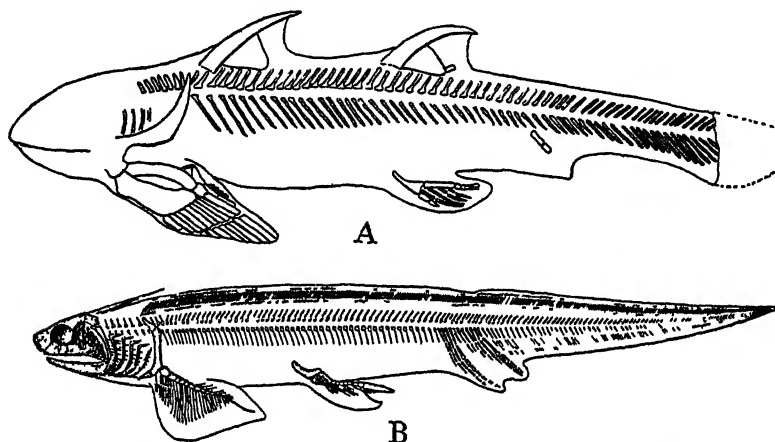


Fig. 3. A. *Tristychius arcuatus*, from Moy-Thomas (1936 *b*). B. *Pleuracanthus sessilis*, after Jaekel (1906). Restoration of fishes in lateral view.

radials, but the pelvic fins are primitive in having a jointed basipterygial axis. The teeth are blunt and there must have been a somewhat crushing dentition.

The pleuracanth (Fig. 3 B) (Broili, 1904; Fritsch, 1889; Jaekel, 1895; Koken, 1889; Reis, 1897) which range from the Carboniferous to the Triassic form a very compact group. The teeth are characteristic being of the "Diplodus" type with a small median and large lateral cusps. The jaws are amphistylic, there is a posterior cephalic spine, and the neurocranium of the typical elasmobranch kind (Romer, 1933, 1937). The unpaired fins are more or less continuous and the tail diphyccercal. The pectoral fins are characteristic in being of the "archipterygial" type having a median jointed axis with both preaxial and postaxial radials. There can be little doubt that this type of fin is derived from the cladoselachian type by the freeing of the basals from the body wall to form the axis and the development of postaxial radials (Smith Woodward, 1898; Moy-Thomas, 1936 *a*). The pelvic fins have a

¹ *Cratoselache* (Smith Woodward, 1924 *b*) is probably another such shark, but is at present not sufficiently well known. It seems improbable that it is an arthrodire as Watson (1934) has suggested. *Rhadamas* (Reis, 1913; Jaekel, 1925) may also be a form of this kind, but requires further investigation.

jointed axis with claspers in the male and are very similar to those of the hybodonts, and as in all primitive selachians there are no vertebral centra.

The Jurassic marks the appearance of the main groups of the modern sharks or Euselachii; but, owing to the scarcity of remains in earlier formations, it is not possible to determine whence they have been derived, and at present speculation is profitless. The chief morphological distinctions of these groups are the presence of centra in the vertebral column, the change from amphistylitic to hyostylitic jaw suspension, and the fusion of the two halves of the pelvic girdle.

Of the modern sharks the Notidani appearing in the Middle Jurassic are the most primitive. Their neurocranium is little changed from the Devonian clado-donts and very similar to those of the hybodonts. The jaws are amphistylitic with long slender hyomandibula except in *Chlamyodelachus*, in which the otic process does not articulate with the neurocranium; in all forms the vertebral centra are incompletely formed. They are unlike any other existing sharks in having a single dorsal fin.

The Heterodonti or cestracionts occur first in the Liassic, and although frequently considered to be hybodonts, have been shown by Brough (1935) to be quite distinct. The vertebral column has well developed centra, the two dorsal fin spines smooth and without posterior denticles, both lying at the same angle and both fins having several radials in their internal skeleton. The neurocranium is also very unlike that of the hybodonts. The early members of the group are amphistylitic and have the teeth more or less unmodified but the modern *Heterodontus* has a crushing dentition and is hyostylitic.

In the Upper Jurassic the three major groups of modern sharks the Scyllioidei, Squaloidei and Rajoidei appear for the first time, and branch into many families in the Cretaceous and Tertiary. All these forms have well developed centra and are hyostylitic. The least specialized of these are the Scyllioidei which form the vast majority of the active fast-swimming carnivorous sharks with a small or entirely reduced spiracle. These occur comparatively rarely in the upper Jurassic, being represented by forms such as *Palaeoscyllium*. In the Cretaceous the lamnids appear, and finally the carcharids in the Tertiary.

The Squaloidei have lost their anal fin and have an enlarged spiracle, characters which suggest they are close to the ancestry of the bottom living rajoids. Members of this group are found in the Upper Jurassic, one of them *Protospinax* still having an anal fin. In the Cretaceous a new type, the pristiphorids, appears having an elongated rostrum with denticles.

The flattened Rajoidei with their ventral gill slits and enlarged spiracles are known in the Upper Jurassic by two forms, which are less modified for bottom living than the majority of the group. In both these, *Squatina* and *Rhinobatus* the pectoral fins are enlarged but do not reach the snout, the body is still fairly rounded, and in the former the gill slits are not completely ventral. In the Cretaceous, the pristids, rajids, trigonids and ptychodonts appear. The pristids or saw-fishes, apart from their rostra, differ little from *Rhinobatus*. The rajids or typical rays are far more modified with huge pectoral fins, which may almost or actually meet in

front of the head. The trigonids, the sting rays and eagle rays, are the most modified family, the pectoral fins meeting in front of the snout, and even forming an anterior cephalic fin. The ptychodonts are confined to the Cretaceous, known only by crushing teeth. In the Tertiary a further specialized group, the torpedinids, occurs, in which the fins have fused with the head to produce a rounded outline and large anterior electric organs are formed.

(b) *Bradyodonti*

In the Upper Devonian, sharks with the typical bradyodont tooth structure make their appearance, and by the Carboniferous a number of fairly well defined groups are distinguishable, which dwindle and become all but extinct by the Permian. Owing to the very fragmentary remains of these forms, separation into groups is not always easy but the following can be distinguished, the Cochliodonti, Holocephali, Petalodonti, Psammodonti, Copodonti. The Edestidi and Chondrenchelydi, which are placed in the bradyodonts, present a special problem and will be dealt with separately.

The cochliodonts are by far the best known of these groups and range from the Devonian to the Permian. In the cochliodonts some of the teeth (Figs. 4 D, 7 F) are always fused into slowly replacing tooth-plates which tend to form a scroll at their outer edge (Smith Woodward, 1892). In some the teeth may be fairly distinct (*Helodus*) (Fig. 4 D), but in most they have become more or less entirely fused into grinding plates. The cochliodonts are seldom known except as teeth and almost certainly represent several distinct families. Among the very few in which any of the anatomy is known, some have the head covered in spines such as the lower Carboniferous *Oracanthus armigerus* (Traquair, 1888b; Smith Woodward, 1915; Moy-Thomas, 1936b) and the Permian *Menaspis* (Dean, 1904, 1906; Weigelt, 1930), whereas others like the Carboniferous *Helodus simplex* (Moy-Thomas, 1936c) are unarmoured.

The anatomy of *Helodus* (Figs. 4 C, D and 5) is fairly well known and is remarkable for the great similarity of its skull with that of the modern *Chimaera* (Fig. 4 A, B). The palatoquadrates are fused with the neurocranium, a condition known as holostyly (Gregory, 1904; De Beer & Moy-Thomas, 1935), and the general shape of the skull and distribution of nerve and blood vessel foramina are as would be expected in a primitive holocephalan. The anterior dorsal fin only has a fin-spine, the pectoral fins are dibasal with a single propterygium and a large metapterygium, and the radials which are much jointed do not extend to the fin margin. The pelvic fins have a single undivided basipterygium and the two halves of the girdle are separate. Although *Helodus* resembles the chimaeroids in these characters, it is more primitive in that the ethmoidal canal is incomplete, there is no evidence of cephalic or anterior pelvic claspers, and the vertebral column has no trace of the calcified rings so characteristic of the Holocephali.

The cephalic spines of *Oracanthus* and *Menaspis* are of particular interest as they resemble in many respects the head spines of the Liassic holocephalan

Myriacanthus, particularly the spines attached to the hind end of the lower jaw which are of very much the same shape in all three fishes. It has long been emphasized (Owen, 1840; Egerton, 1872; Jaekel, 1891; Smith Woodward, 1921) that the teeth of the Holocephali, particularly those of *Myriacanthus* are very

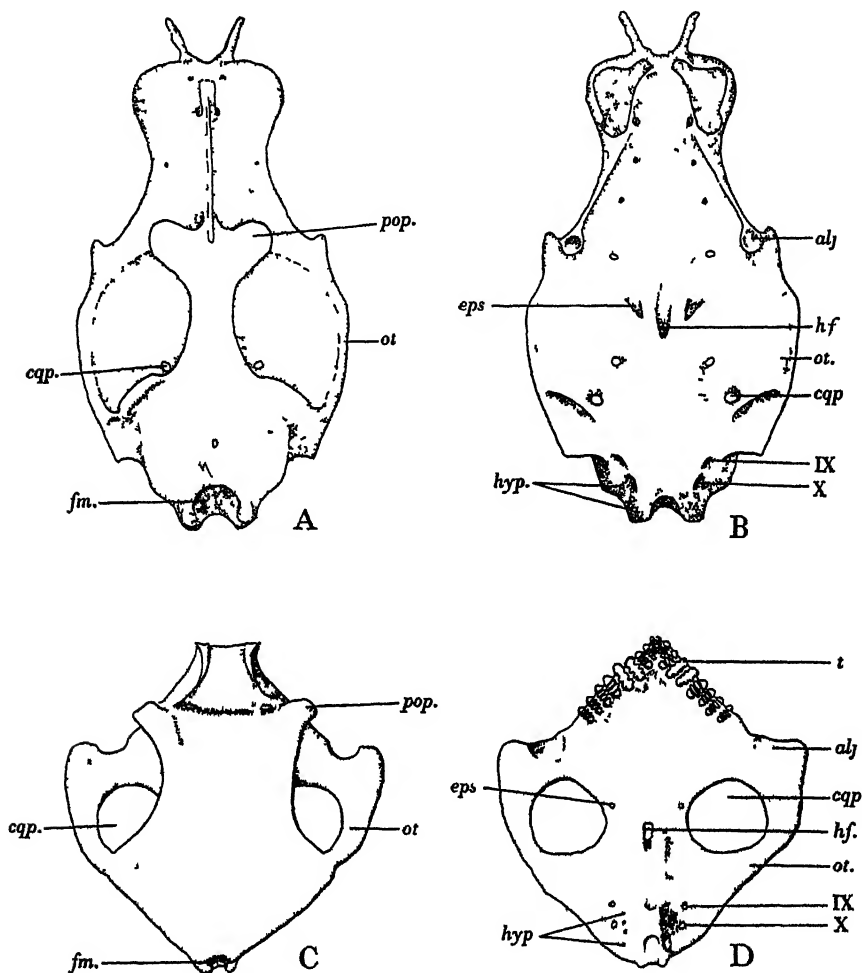


Fig. 4. A. Dorsal view of skull of *Chimaera collieri*. B. Ventral view of same. Both after Allis (1917), redrawn. C. Dorsal view of skull of *Helodus simplex*. D. Ventral view of same. Both from Moy-Thomas (1936c). *alj* articular surface for the lower jaw; *cqp* cranioquadrate passage; *eps* foramen for the efferent pseudobranchial artery; *fm*. foramen magnum; *hf*. hypophysial foramen; *hyp*. foramina for the hypoglossal nerves; *ot*. otic process; *pop* preorbital process; *t*. teeth; IX, foramen for the glossopharyngeal nerve; X, foramen for the vagus nerve

similar in general appearance to those of the coeliodonts. This resemblance is even more obvious when the form of the teeth of *Myriacanthus* is compared with that of *Oracanthus*, which in addition has a series of small teeth in front of the upper, but not the lower, tooth plates as in *Myriacanthus* but not other Holocephali.

Although the microscopic structure of the teeth of modern holocephalans is rather difficult to compare with that of the coeliodonts, the teeth of *Myriacanthus* are very similar, differing only in the more branched nature of the vertical dentinal tubules. There can therefore be very little doubt that the Holocephali are the direct descendants of the coeliodonts.

The Holocephali first appear in the Triassic and continue to the present day. The Liassic forms *Squaloraia* and *Myriacanthus* differ only in detail from the modern ones, the group being very constant in structure. The chief characteristics of the Holocephali are the crushing teeth, the holostylic jaws, the presence of only a pro- and metapterygium in the pectoral fins, and of cephalic and anterior and posterior pelvic claspers; moreover in most there is only an anterior dorsal fin spine, and there are calcified rings in the vertebral column greater in number than the vertebrae. The Holocephali are thus the only living survivors of the large Palaeozoic group of bradyodonts.

The petalodonts, which range from the Carboniferous to the Permian, are known from little else but teeth, and it is only in the Carboniferous *Ctenoptychius*

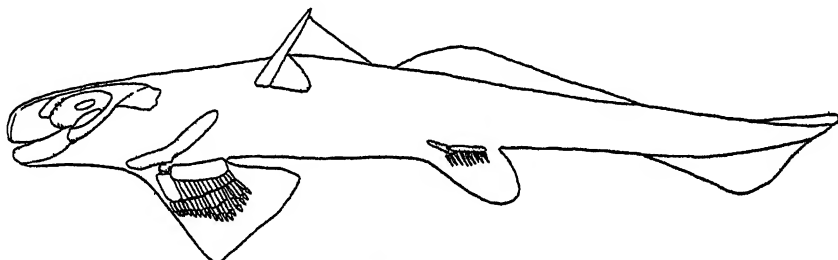


Fig. 5. *Helodus simplex*, after Moy-Thomas (1936c). Redrawn.
Restoration of fish in lateral view

(Smith Woodward, 1932) and the Permian *Janassa* (Fig. 7 G) (Jaekel, 1899) that anything of the body structure is known. The teeth of the petalodonts are antero-posteriorly compressed and closely arranged in longitudinal and transverse rows. The teeth in some, e.g. *Climaxodus* (Smith Woodward, 1919), may function simultaneously or one row at a time as in *Janassa*. Little more can be said of the body except that it is dorsoventrally compressed and very ray-like in general shape with enlarged pectoral fins. The method of attachment of the palatoquadrates is unknown, but there is no evidence inconsistent with the view that the Petalodonts were holostylic.

The psammodonts (Fig. 7 E) are confined to the Carboniferous, and are known only by their teeth which are flattened grinding plates arranged in two longitudinal series along the symphysis of the jaws. These teeth are of particular interest as they are apparently asymmetrical (Smith Woodward, 1921), those of one side being much larger than those of the other.

The copodonts (Fig. 7 D) (Davis, 1883; Hussakof & Bryant, 1919) are also known only by their teeth, and occur in the Upper Devonian and Carboniferous. The teeth are apparently bilaterally symmetrically arranged on the symphysis of

the jaws in a single antero-posterior series. The teeth are embedded in a plate of highly vascular dentine. *Acmoniodus* shows two such teeth of different sizes embedded in a pentagonal plate of dentine.

The edestids (Fig. 7 A, B, C) include a number of Carboniferous and Permian sharks known only by their teeth, and a few other very fragmentary remains. The teeth on the rami of the jaws are not very specialized for crushing and not particularly slowly replacing. The symphyseal teeth characteristic of the group are prominent, and in some, e.g. *Agassizodus* (Fig. 7 A) (Nielsen, 1932) and *Edestus* (Fig. 7 B) (Hay, 1912; Woodward, 1916b), are shed at intervals during life while in others they persist throughout life to form a spiral, e.g. *Helicoprion* (Fig. 7 C) (Karpinsky, 1899). The edestids had been generally considered to be selachians allied to the hybodonts, until Nielsen (1932, 1935) pointed out that in some of the less specialized genera (*Agassizodus* and *Fadenia*), the microscopic structure of the teeth was typically bradyodont and that they should be included in this group. Karpinsky, however, has described a very "enamel"-like outer layer in *Helicoprion*, and since in many other forms the microscopic structure of the teeth is unknown it is perhaps

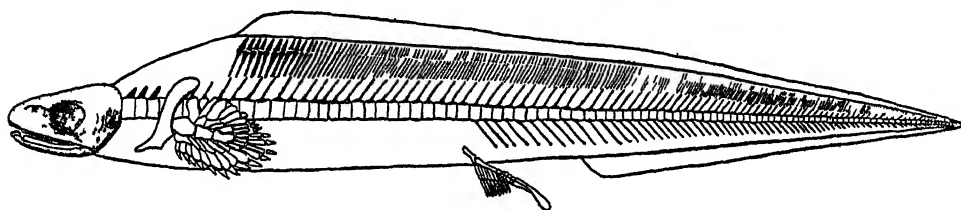


Fig. 6. *Chondrenchelys problematica*, from Moy-Thomas (1935a)
Restoration of fish in lateral view.

dangerous to conclude that all forms included in this group are bradyodonts. If tooth structure is, as I believe, of value in classification, it seems possible that the edestids are a heterogeneous collection of sharks, some being bradyodonts, others selachians. Only further research and better material can decide this question.

The Upper Devonian and Lower Carboniferous elongated obtuse teeth, named *Orodus* (Fig. 7 H) have been frequently compared with the teeth of the edestid *Agassizodus*, but more recently they have been included by most writers in the hybodonts, and certain *Ctenacanthus*-like spines have been believed to be associated with them (Newberry, 1875). Nielsen (1932) has shown, however, that *Orodus ramosus* has a microscopic structure of the bradyodont type, it therefore seems probable that they are closely related to the less specialized edestids. On the other hand all teeth termed *Orodus* may not have had this microscopic structure, and some may possibly belong to otherwise unknown families of the Selachii.

The chondrenchelyds known by a single genus and species *Chondrenchelys problematica* (Fig. 6) (Moy-Thomas, 1935, 1936) from the Lower Carboniferous in many ways resemble the pleuracanth. Since the structure of the teeth appears to be of the bradyodont type, this resemblance, however, seems probably due to convergence. It would hardly be surprising to find such convergence between

two groups which have been derived from a common ancestor. The body is elongated, the unpaired fins apparently continuous, the tail diphycercal and the pectoral fin has an axis of three joints with both preaxial and postaxial radials, resembling the archipterygium of the pleuracanth. All these characters have obviously been evolved in the two groups in connexion with a similar mode of swimming. The pelvic fins are primitive in having a jointed basipterygial axis, and the body is

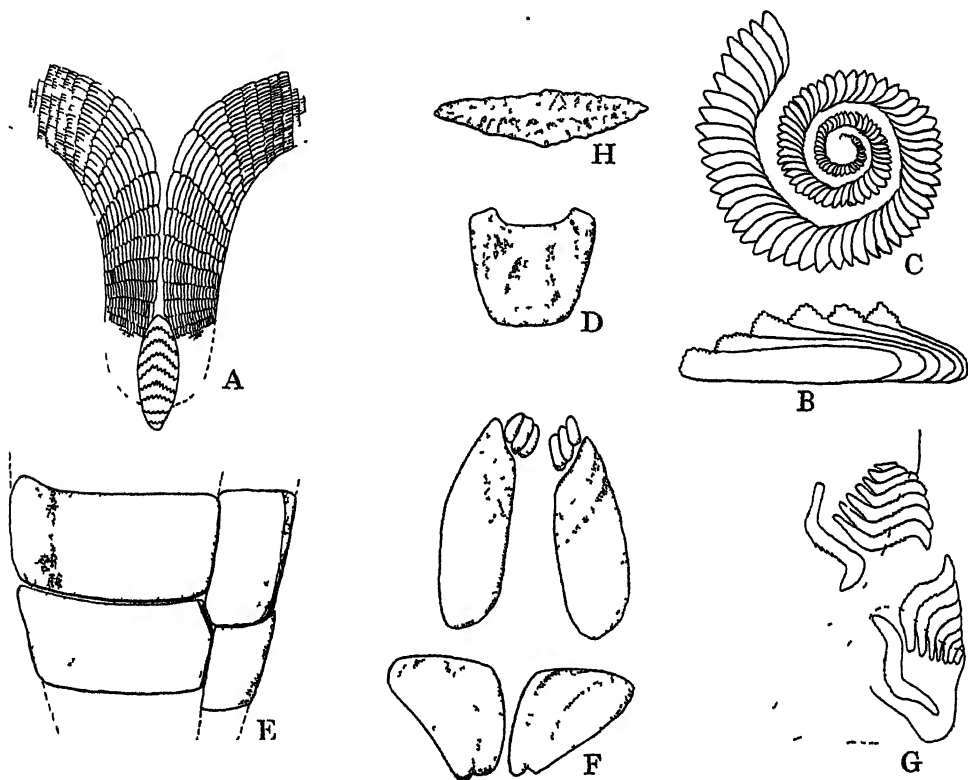


Fig. 7 Bradyodont teeth A *Agassizodus* sp., after Nielsen (1932) B. *Edestus crenulatus*, after Hay (1912). C *Helicoprion bevanowi*, after Karpinsky (1889). D. *Copodus spatulus*, after Davis (1883) E *Pseudonodus rugosus*, after Smith Woodward (1921). F. *Oracanthus armigerus*, after Moy-Thomas (1936b). G *Janassa butimnosa*, after Jaekel H *Orodus ramosus*.

naked except for a few scattered denticles. The teeth are crushing tooth-plates, two relatively large pairs meeting one another in the middle line in both upper and lower jaws. Anteriorly to these plates are a number of smaller plates. The jaw suspension is almost certainly holostylic. The vertebral column of *Chondrenchelys* is interesting in having calcified rings in it, one to each neural arch. These latter characters coupled with the tooth structure seem sufficient for considering *Chondrenchelys* to be a specialized bradyodont group.

Probably related to *Chondrenchelys* is *Eucentrurus* a lower Carboniferous fish known from a single specimen *E. paradoxus* (Traquair, 1905; Moy-Thomas, 1937).

Unfortunately *Eucentrurus* is not well known, and although a relatively large bradyodont tooth plate is distinguishable the arrangement of the dentition is unknown. The vertebral column had calcified rings very like those of *Chondrenchelys*. Although limb girdles are present no trace of the fins is preserved. The body differs from *Chondrenchelys*, being covered with numerous denticles which are enlarged into spinelets posteriorly.

III. CLASSIFICATION OF THE ELASMOBRANCHS

The foregoing account of the evolution of the Elasmobranchs may be summarized in the following classification:

Subclass. ELASMOBRANCHII.

Division 1. SELACHII.

Order 1. PLEUROPTERYGII.

Suborder 1. Cladoselachii.

E.g. *Cladoselache*, "*Cladodus*" *wildungensis*, "*C.*" *hassiacus*, "*C.*" *neilsoni*, *Denaea*, *Symmorium*.

Suborder 2. Ctenacanthii.

E.g. *Ctenacanthus*, *Goodrichia*.

Order 2. PROTOSELACHII.

Suborder 1. Hybodontii.

E.g. *Hybodus*, *Lissodus*, *Carinacanthus*, ? *Petrodus*.

Suborder 2. Tristychii.

E.g. *Tristychius*.

Order 3. EUSELACHII.

Suborder 1. Notidani.

E.g. *Notidanus*, *Chlamydoselachus*.

Suborder 2. Heterodonti.

E.g. *Palaeospinax*, *Synechodus*, *Cestracion*.

Suborder 3. Scyllioidei.

E.g. *Scylliorhinus*, *Orectolobus*, *Lamna*, *Rhinodon*, *Carcharias*.

Suborder 4. Squaloidei.

E.g. *Protospinax*, *Pristiophorus*, *Acanthias*.

Suborder 5. Rajoidei.

E.g. *Squatina*, *Raia*, *Rhynobatus*, *Torpedo*, *Ptychodus*.

Order 4. PLEURACANTHODEI.

E.g. *Pleuracanthus*, *Xenacanthus*, *Dicranodus*.

Division 2. BRADYODONTI.

Order 1. EUBRADYODONTI.

Suborder 1. Cochliodonti.

E.g. *Helodus*, *Oracanthus*, *Cochliodus*, *Menaspis*.

Suborder 2. Holocephali.

E.g. *Myriacanthus*, *Squaloraia*, *Chimaera*.

Suborder 3. Petalodonti.

E.g. *Climaxodus*, *Ctenoptychius*, *Janassa*.

Suborder 4. Psammodonti.

E.g. *Psammodus*.

Suborder 5. Copodonti.

E.g. *Acmoniodus*, *Copodus*.

Suborder 6. Edestidi.

E.g. *Edestus*, *Agassizodus*, *Orodus*, ? *Helicoprion*.

Order 2. CHONDRENCHELYDI.

E.g. *Chondrenchelys*, ? *Eucentrurus*.

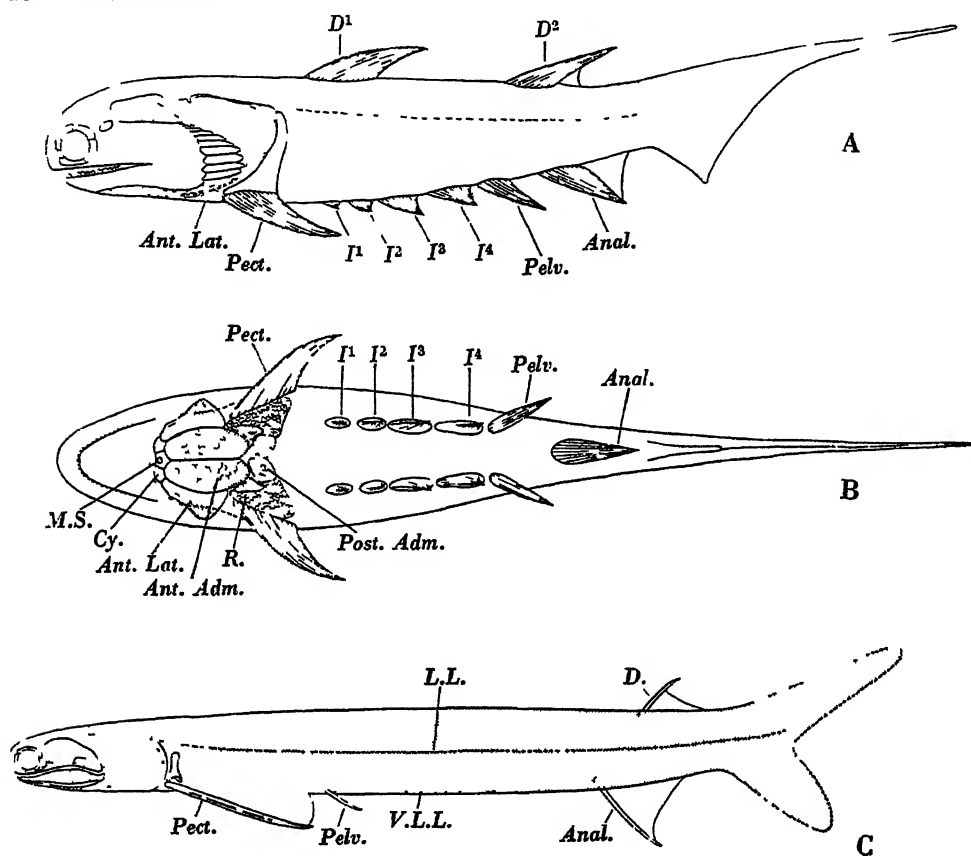
IV. THE RELATIONSHIPS OF THE ELASMOBRANCHII WITH THE PLACODERMI

As has already been pointed out, the placoderms have been of recent years fairly generally considered to have some close relationship with the elasmobranchs. Watson (1937) has, however, challenged the entire existing view of the early evolution of the Gnathostomes, and has brought forward very strong evidence to show that the placoderms and elasmobranchs have no close affinity with one another. Since previous writers have been at variance as to the relationship of individual placoderm groups, the Acanthodii, Antiarchi, Arthrodira, Macropetalichthyda and Rhenanida, both with one another and with the elasmobranchs, it is considered desirable here to give some discussion of each group separately.

(a) *Acanthodii* (Fig. 8)

The Acanthodii have been a much neglected group in recent years, but Watson (1937) has given an exhaustive and revolutionary account of them, which has profoundly altered current views on their anatomy and relationships. The acanthodians appear in the Upper Silurian, being the oldest known gnathostomes, and continue to the Lower Permian. The general body shape is somewhat similar to that of the elasmobranchs with one or two dorsal fins, an anal fin, and a heterocercal tail. Between the paired pectoral and pelvic fins in primitive forms (*Climatius*, Fig. 8 A, B) there is a series of additional paired fins. All the fins except the caudal have an anterior fin spine, which in early forms (*Climatius*) is broad and thick without any prolongation into the body, being little more than an enlarged scale. In the later forms (*Acanthodes*, Fig. 8 C) the spines are laterally compressed with a considerable part of their length embedded in the body. The internal skeleton of the fins where known consists of short concentrated radials, ceratrichia, and an external coating of minute closely fitting ganoid scales (? lepidotrichia), similar to the body scales which in many ways resemble the "ganoid"

scale (Goodrich, 1907).¹ The shoulder girdles consist of two parts, an endoskeletal scapula and sometimes a coracoid part, and in early forms an irregular series of dermal ossifications.



Text-fig. 8. A. *Clamatus reticulatus*. Reconstruction of fish in lateral view. B. In ventral view. C. *Acanthodes* sp., restoration of fish in lateral view. All from Watson (1937). A, B. Anal. anal fin; Ant. Adm. anterior admedian dermal bone of shoulder girdle; Ant. Lat. anterior lateral of shoulder girdle; Cy. cylindrical dermal bone of shoulder girdle; D¹ and D², dorsal fins; I¹-I⁴, intermediate spines; M.S. median dermal bone of shoulder girdle; Pect. pectoral fin spine; Pelv. pelvic fin spine; Post. Adm. posterior admedian of shoulder girdle; R. ridged dermal bone of shoulder girdle. C. Anal. anal fin; D. dorsal fin; L.L. main lateral-line; Pect. pectoral fin; Pelv. pelvic fin; V.L.L. ventral lateral-line.

The neurocranium is tropibasic, and has several perichondral ossifications, small anteriorly placed nasal capsules lying close together, and a long occipital region. The palatoquadrates do not meet in the middle line, may be formed of one or several ossifications, and apparently did not primitively have an otic process. Meckel's cartilage may also be ossified as a single or several bones. Where teeth are present on the jaws they usually form a whorl, showing "elasmobranch"

¹ Gross (1938) describes scales of this nature associated with the Devonian Cladodont, *Protacrodus vetustus*. However, it is very uncertain that these scales belong to *Protacrodus*.

replacement. The mandibular arch forms an operculum, which increases in importance during the evolution of the group, at the expense of the smaller hyoidean and branchial opercula. The hyoid arch is separated from the mandibular by a greatly elongated gill slit, and with the possible absence of a pharyngohyal is ossified in four parts, and is >-shaped as are the succeeding branchial arches. The skull is covered with numerous small dermal bones of varying patterns, which extend back on to the opercula, so that primitively there are dermal ossifications on all the branchial arches. The main lateral line of the body runs between two rows of scales on to the head, where the pattern is rather similar to that of the arthrodires, but in some ways more primitive. A special opercular canal is developed in *Acanthodes*.

The main characters which have been responsible for the inclusion of the Acanthodii in the elasmobranchs have been the fins with spines, heterocercal tail, ceratotrichia, endoskeletal shoulder girdle, tooth replacement, and the lateral line running between the scales.

The internal skeleton of the fins has become, however, far more advanced in being formed of concentrated radials, before the appearance of the earliest elasmobranch. Similar fin spines are paralleled in the ostracoderms. The "elasmobranch" tooth replacement is apparently a primitive character, occurring in other placoderms. The early forms appear to have had a dermal girdle as well as an endoskeletal one, and although the lateral line runs between the scales, the acanthodian scales are not placoid denticles. On the other hand the presence of a fully developed gill slit behind the mandibular arch,¹ and the mandibular operculum are characters suggestive of a more primitive group than the elasmobranchs. The neurocranium is platybasic and only superficially calcified with prismatic granules in the elasmobranchs, whereas it is tropibasic and with perichondral bones in acanthodians. Unlike the elasmobranch condition the nasal capsules are small and close together and the otic region very long. The palatoquadrate and Meckel's cartilages of elasmobranchs do not ossify in several pieces and the branchial arches are <-shaped and not >-shaped as in acanthodians. The sensory canals of the head are more like those of the arthrodires than the elasmobranchs, and the presence of dermal and perichondral bones also suggests arthrodire affinity.

It may, therefore, be concluded that the acanthodians are in fact not very close relatives of the elasmobranchs, but show more affinity with the Arthrodira.

(b) *Macropetalichthyda* (Fig. 9)

Our knowledge of the Macropetalichthyds was limited to the neurocranium (Stensiö, 1925), and dermal head bones (Stensiö, 1925; Gross, 1933 b) of the Middle and Upper Devonian *Macropetalichthys* and *Epipetalichthys* and a description of the body by Broili (1933 a, b) of three Lower Devonian fishes "*Macropetalichthys* (?) *prumiensis*" (Fig. 9 B), *Stensiöella* and *Nessariostoma*, which have elasmobranch-like fins and no pectoral shield. Recently, however, Gross (1937 b) has shown that

¹ Unless Jaekel (1926) is correct in his interpretation of *Pleuracanthus sessilis*.

the Lower Devonian fish *Lunaspis*, which has both a pectoral shield, pectoral fin spines and a scale-covered body is a macropetalichthyid not an acanthaspide Arthrodire as Broili (1929, 1930a) supposed. He has consequently pointed out that the Macropetalichthyda consist of two groups, the Petalichthyda, including *Macropetalichthys*, and *Lunaspis*, and the Stegoselachii including "*Macropetalichthys* (?)

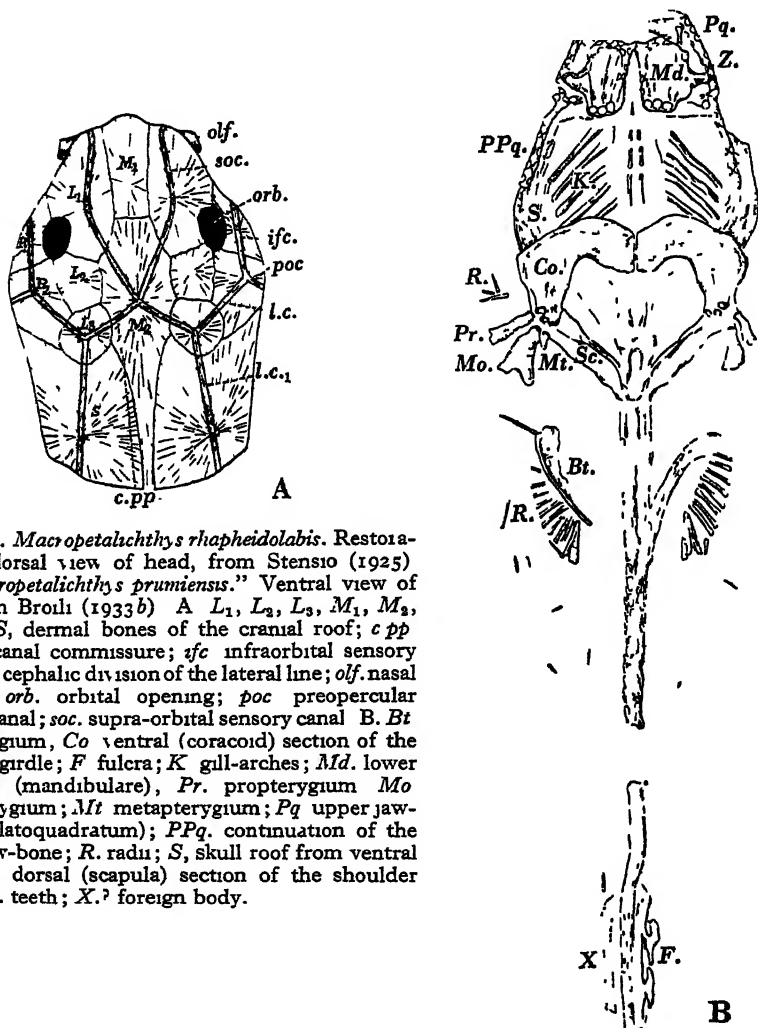


Fig. 9 A. *Macropetalichthys rhapsoidolabis*. Restoration of dorsal view of head, from Stensio (1925) B. "*Macropetalichthys prumiensis*." Ventral view of fish, from Broili (1933b) A. L_1 , L_2 , L_3 , M_1 , M_2 , P_1 , P_2 , S , dermal bones of the cranial roof; $c.pp$ sensory canal commissure; ifc infraorbital sensory canal; $l.c$ cephalic division of the lateral line; olf nasal capsule; orb orbital opening; poc preopercular sensory canal; soc supra-orbital sensory canal B. Bt basipterygium, Co ventral (coracoid) section of the shoulder girdle; F fulcrum; K gill-arches; Md lower jaw-bone (mandibular), Pr propterygium Mo mesopterygium; Mt metapterygium; Pq upper jaw-bone (palatoquadratum); PPq continuation of the upper jaw-bone; R radius; S skull roof from ventral side; Sc dorsal (scapula) section of the shoulder girdle; Z teeth; X ? foreign body.

prumiensis", *Stensioella* and *Nessariostoma*. This has been supported by Heintz (1937) and appears undoubtedly to be the correct interpretation, nevertheless the affinities of the Stegoselachii are still rather obscure. Broili's figures and descriptions of the dermal bones of the skull in "*M. (?) prumiensis*", *Stensioella*, and *Nessariostoma*, although the state of preservation of the material prevents their being entirely satisfactory, show a condition strikingly like that of *Macropetalichthys*. From the

point of view of affinity with the elasmobranchs, it matters little whether the stegoselachians are closely related to the petalichthyds, since each can be treated independently.

The petalichthyds have a neurocranium roofed by dermal bones arranged in a definite pattern (Fig. 9 A). The neurocranium is ossified and very like that of the acanthaspid Arthrodires. Stensio (1925) compared the neurocranium of *Macropetalichthys* with that of the elasmobranchs, demonstrating a number of points of supposed affinity, but all these have been disposed of by Watson (1937) on the grounds that they are primitive, or convergent features, or withdrawn by Stensio himself (1934 *b*). The most noteworthy of these is the platybasia of the skull, which Watson considers to be a convergent character, developed in connexion with the dorsal eyes of a bottom-living form, rather than one of affinity. Watson has further pointed out that they were probably operculate, since the preopercular sensory canal as in Arthrodires passes backwards and outwards being cut off abruptly by the margin of the head shield and in all probability continuing on to an unossified operculum. Gross's interpretation of *Lunaspis*, showing the existence of a pectoral shield, is overwhelming evidence of close arthrodire affinity.

In the Stegoselachii, only "*Macropetalichthys prumiensis*" (Fig. 9 B) need be discussed, as so little is known of the other two forms. The skull roof is bony, and the sensory canals, as far as can be seen, appear to be similar to those of *Macropetalichthys*. Watson (1937) has also demonstrated the very great probability of the hyoid arch playing no great part in the jaw suspension. The body shape and fin structure is rather like that of the elasmobranchs. The mouth is ventral, the teeth although crushing appear to be little more than modified scales, and there are five branchial arches. The pectoral girdle is very elasmobranch-like in having both scapular and coracoid portions, the latter meeting in the middle line. The pectoral fin is tribasal and the pelvic has an unsegmented basipterygium. However, as Watson has emphasized, the fins correspond to those of *specialized* elasmobranchs, and are far more developed than those of the primitive Upper Devonian Chondrichthyes, and most important of all, similar pectoral girdles, fins and branchial arches are found in acanthodians. The condition of the teeth is obviously primitive, that of the mouth convergent in connexion with bottom-living. These points, and the fact that the Stegoselachii are apparently bony and elasmobranchs entirely cartilaginous, show that there is little close affinity between the two. The general appearance of the shoulder girdle, and the nature of the dermal skeleton would suggest that the Stegoselachii are related to the Rhenanida.

(c) *Arthrodira* (Fig. 10)

The Arthrodira, consisting of the Lower Devonian acanthaspids (Fig. 10 A) (Heintz, 1929), and the Middle and Upper Devonian coccosteids (Fig. 10 B, C) (Heintz, 1931, 1932 *a, b*, 1933; Gross, 1932), ptyctodonts (Watson, 1934, 1938), and phyllolepid (Stensio, 1934 *a*, 1936), have as their distinguishing features a head covered with dermal bony plates of a definite pattern (Fig. 10 B), somewhat similar to that of the macropetalichthyds, and a large dermal pectoral carapace movably

articulated with it. The body is tapering with a dorsal, possibly an anal, and a diphyccercal caudal fin. The internal skeleton of the dorsal fin is composed of two rows of radials (axionots and baseots) and the notochord is persistent. Pectoral fins are usually not preserved, and in the primitive acanthaspids (Fig. 10 A) are represented by long spines rigidly fixed to the carapace, which are reduced to smaller movable spines or disappear in later forms. However, the presence of radials behind the spine in some forms has led Heintz (1938) to believe that pectoral fins were present in all Arthrodira. The pelvic fins are small with a girdle pierced for

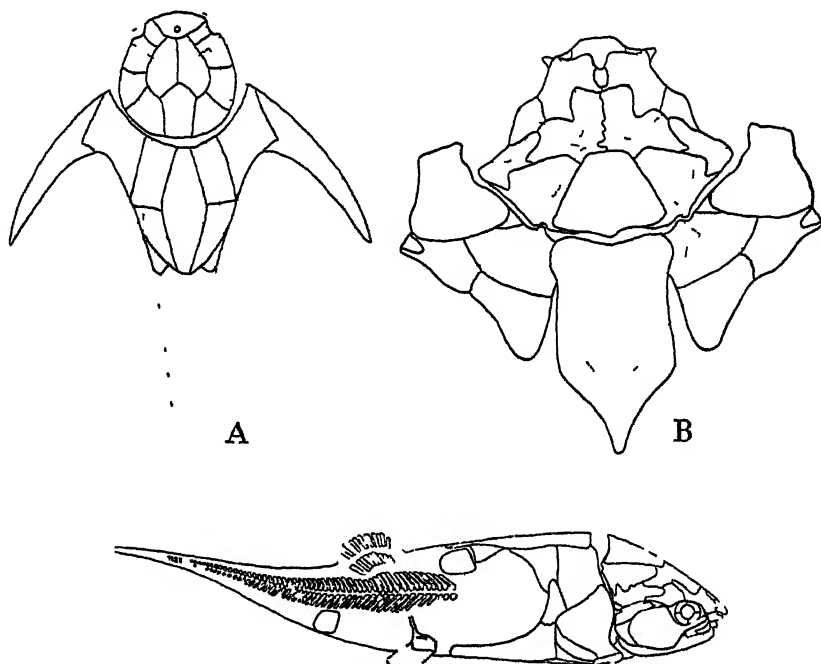


Fig. 10. A. *Acanthaspis* sp. restoration of fish in dorsal view after Heintz (1929). B. *Coccosteus decipiens*. Restoration of head and pectoral shield in dorsal view from Watson (1935). C. *Coccosteus decipiens* from Watson (1935).

the diazonal nerves, and a short row of radials. The body appears generally to have been naked, but in a few there are some bony scales.

The neurocranium in the primitive acanthaspids (Stensiö, 1934*b*; Gross, 1937*b*) is lined by a continuous sheet of perichondral bone, whereas in the coccosteids, *Leiosteus*, *Pholidosteus* (Stensiö, 1934*b*), and *Coccosteus* (Hills, 1936) it is less well ossified and formed of several bones. The acanthaspid neurocranium is very like that of *Macropetalichthys*, and the unpaired ossifications of *Pholidosteus* very like that of *Acanthodes*. In all cases the nasal capsules are small, lying close together in the middle line, and the occipital region relatively very long. In *Pholidosteus* the palatoquadrate and in both *Pholidosteus* and *Leiosteus* Meckel's cartilage are ossified

in two parts as in acanthodians. Watson (1934, 1937) has demonstrated that as in the macropetalichthyds there is a very great probability that an operculum was present. The sensory canals are somewhat similar to those of the acanthodians, and the teeth have been shown by Watson to show signs of "elasmobranch" replacement.

The supposed elasmobranch affinities of these fishes rested very largely on the similarity of the neurocranium with that of *Macropetalichthys*, but this has been shown by Watson not to be of the elasmobranch type. The nature of the unpaired bones of the neurocranium, the position of the nostrils, and the long occipital region are also typical of the acanthodians. Watson believes the pectoral spines are

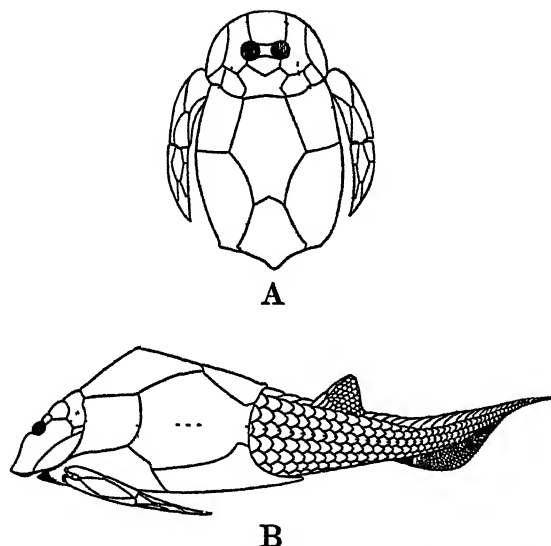


Fig. 11. A. *Pterichthyodes milleri*. Restoration of skull and pectoral shield in dorsal aspect.
B. *Pterichthyodes milleri*. Restoration of entire fish. All from Watson (1935).

directly comparable with those of acanthodians, and to have been derived from fins of this type. The pectoral girdle, the double nature of the ossifications of the mandibular arch, the operculum, and the lateral line system all show placoderm and not elasmobranch affinity.

The Arthrodira, therefore, may be said to show little or no close affinity with elasmobranchs, but sufficient characters of an acanthodian and macropetalichthyid type to suggest that all are members of a natural group.

(d) *Antiarchi* (Fig. 11)

The Antiarchi (*Pterichthyodes*—Middle Devonian, *Bothriolepis*—Upper Devonian, Traquair (1894–1913); Forster-Cooper, 1934; Stensiö, 1932; Gross, 1932) have a bony head shield (Fig. 11) with a thoracic carapace hinged to it, and true jaws. The eyes and nostrils are dorsal and close together on the top of the head,

which probably accounts for most of the discrepancies in arrangement between their dermal bones and those of the Arthrodira. There is no doubt that an operculum is present. The body is covered with bony scales, there is a single dorsal fin, and in *Bothriolepis* probably paired pelvic fins. The pectoral fins are peculiar, being spine-like, usually with a joint, and covered by a number of bony plates. In all probability these fins are a modification of the arthrodiran fin spine.

From this account there can be no reasonable doubt that the antiarchs are close relatives of the Arthrodira, and not of the elasmobranchs.

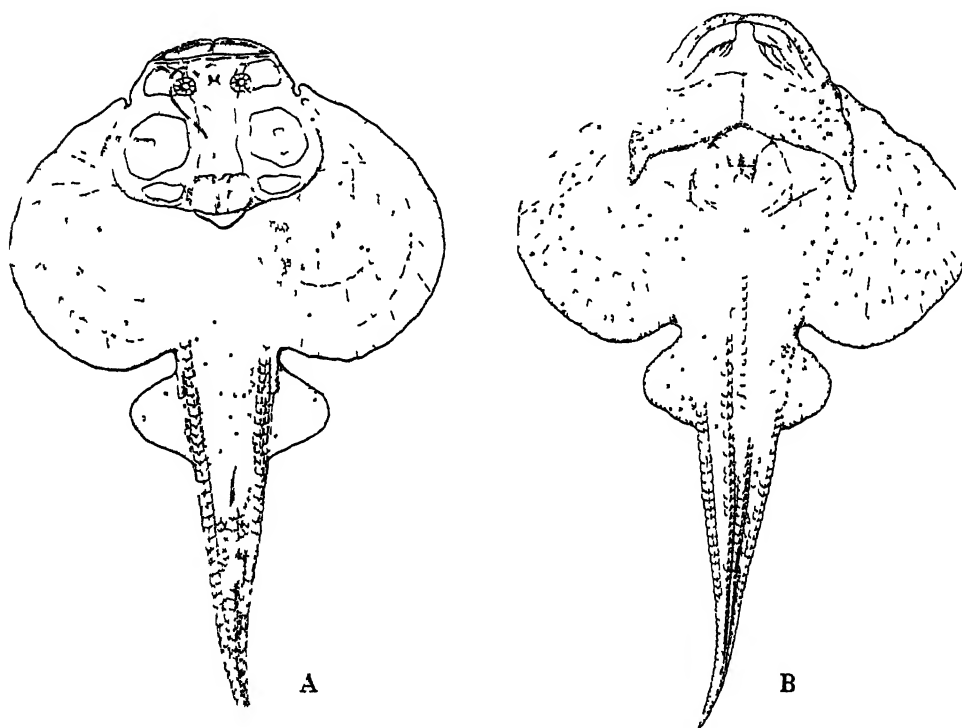


Fig. 12 *Gemündina sturtzi*. Restoration of the fish A, in dorsal, B, in ventral view. Both from Broili (A, 1933; B, 1930).

(e) *Rhenanida* (Fig. 12)

Only one form, the lower Devonian *Gemündina* (Broili, 1930, 1933) included in this group is well known; others *Jagorina* (Holmgren & Stensiö, 1936) and *Asterosteus* (Newberry, 1875; Smith Woodward, 1934) are known only from the neurocranium. Superficially *Gemündina* is extremely ray-like in appearance, being dorsoventrally flattened with expanded pectoral fins, the radials of which extend to the fin margin, and in having a tapering body. A small spine marks the presence of a dorsal fin. The mouth and nostrils, however, are dorsal, the latter close together near the middle line. The teeth are small and conical with "elasma-

branch" replacement. The neurocranium of the Rhenanida is more like that of the elasmobranchs than in any of the preceding groups, but lined with perichondral bone. But, as Watson has pointed out, this resemblance is almost certainly due to convergence in a ray-like form. The head has a few dermal bones, the sensory canals as far as described are typically arthrodiran, and there was almost certainly an operculum.

There is according to Watson (1937) a pectoral shield in *Gemündina* comparable to that of an arthrodire; but with fewer plates, as in the ptyctodonts. If Watson is correct in believing a pectoral shield to have been present, and this seems most probable as Professor Stensiö has demonstrated to me the remains of a similar shield in *Jagorina*, there can be little doubt of the close arthrodiran affinity of the Rhenanida.

V. SUMMARY AND CONCLUSIONS

1. The early evolution of the elasmobranchs (Chondrichthyes) show that the group arose in the Upper Devonian, and that *Cladoselache* must have been very similar to the ancestral form. The elasmobranchs very early in their history became divided into two groups, the Selachii and Bradyodonti, distinguishable by their tooth structure.

2. The Selachii are shown to have arisen directly from the Cladoselachii; the Ctenacanthi being a group which shows many characters intermediate between them and the later sharks, particularly the Hybodontii. Although there are indications of the existence of many other selachian groups descended from forms like the Cladoselachii, only the Tristychii and Pleuracanthodei are at all well known. The latter are a line of evolution distinct from other selachians, whereas from groups like the Trisychii and the Hybodontii, the modern Notidani, Heterodonti, Scyllioidei, Squaloidei, and Raiioidei must have arisen.

3. The origin of the Bradyodonti is less clear, but must have been from some early selachian stock. The group includes the Holocephali and their Palaeozoic ancestors the Cochlodonti, and the less well known Petalodonti, Psammodonti, Copodonti, and probably some of the Edestidi. The Chondrenchelydi seem to be early bradyodonts which convergently resemble the pleuracanthi.

4. The elasmobranchs form a concise and well defined group, having no very close relationship with the earlier placoderms (Acanthodii, Arthrodira, Macropetalichthyda, Antiarchi, Rhenanida), which are at a lower grade of evolution, forming a diverse group but with many characters in common with one another. Probably all these groups had the hyoidean gill slit completely developed (Watson, 1937).

ACKNOWLEDGEMENT

I would like to express my thanks to Prof. Goodrich for reading this manuscript, and for many helpful criticisms.

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THE ULTRASTRUCTURE OF THE NERVE AXON SHEATH

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(Received 30 March 1938)

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I. INTRODUCTION

NERVE theory is rapidly approaching the point at which an understanding of the molecular organization of the conducting substratum is desirable. Most modern theories of nerve action assume that the phenomena of excitation and propagation involve molecular adjustments at certain interfaces, resulting in changes in capacitance or resistance and in current flow through certain regions. No definite localization of the active physical substratum in terms of actual nerve structures is usually implied in such theories, nor need it be for this method of approach. Nevertheless, it is obvious that the capacitances, resistances, permeability of the "membrane", etc. are determined by specific orientations and peculiar modes of packing of the constituent structural molecules involved. Hence, if it is possible eventually to describe the molecular construction of the various nerve components, this information may lead to a solution of the mechanism which is unique for nerve, a possibility not likely to be achieved by the construction of any number of physical-chemical models, no matter how ingenious they may be or how nearly they may come to reproducing actual nerve phenomena.

The great majority of peripheral and central fibres, both of vertebrates and invertebrates, are enclosed in well defined sheaths, and these sheaths show their highest degree of differentiation in those fibres which are capable of conducting the impulse with greatest frequency and velocity. These facts suggest that the sheath is of considerable importance in the propagation of the nervous impulse. From a structural point of view, data bearing upon this question deal with the relationship of the sheath and its environment, particularly other fibre components, and with the fine structure of the sheath itself. In this review certain aspects of these problems will be considered in detail.

With regard to the relationship of the sheath to other fibre components, little unanimity exists with respect to some of the most essential questions. An example in point is the problem of the physical relationship between the axon and the sheath. Some conclude that the myelin is an inclusion laid down within the protoplasmic framework of the sheath cell. According to this view the myelin substance is enclosed by the external neurilemma and the internal axiolemma membranes, which meet and fuse at the nodes to form an internodal unit (for a review see Maximow & Bloom, 1930). However, microdissection experiments reveal no evidence of an axiolemma membrane but instead indicate an organic union between the myelin and the axis cylinder, the inference being that the myelin belongs structurally and functionally to the axon (de Renyi, 1929*a*). This view is supported by Speidel's (1933) experiments with growing tadpole nerves.

Another approach to this problem is that of a study of the developmental mechanics of sheath formation. It is probable that in vertebrates the sheath is laid down upon the developing axon under the influence of certain ectodermal cells, the Schwann cells, which are believed to have their origin in the dorsal portion of the neural crest. In the central nervous system the functional analogue of the Schwann cell is presumably the oligodendrocyte (del Rio Hortega, 1928; Linell & Tom, 1931; but see also Plenk, 1934). Regarding the details of the process by means of which the "myelin" is laid down under the influence of the sheath cell, Speidel (1933, p. 67) suggests that the lipoids supplied from tissue fluids are built up in the myelin sheath segment through the co-operative metabolic activity of the sheath cell and the axon. He finds (Speidel, 1935*a, b*) that the myelin formation originates near the sheath cell nucleus and proceeds by continuous extension away from the nucleus, and that the "premyelin substance" is isotropic between crossed Nicol prisms, anisotropy appearing and increasing with growth and extension of the segment. Since few experimental details are given, however, it is not clear whether sufficiently sensitive optical methods were used or whether allowance was made for the thickness of the object in estimating its anisotropy. A careful study of this problem might lead to significant conclusions as to the fine structure of "myelin" in various stages of its development.

Even more difficult to investigate by classical methods is the fine structure of the sheath. In the case of the vertebrate fibre's myelin sheath these methods have revealed a variety of configurations depending upon the particular fixing and staining techniques employed. Among these may be mentioned the Schmidt-

Lantermann incisures which demarcate the internal cylindroconic segments, Golgi funnels with spiral apparatus of Rezzonico, spiny bracelets of Nageotte, the neurokeratin network, and the sheath cell with its inclusions. Appropriate methods may also show each cylindroconic section to be composed of lipoidal rodlets (mitochondria of Nageotte) extending from the inner to the outer surface of the segment and disposed at an angle from the normal to the fibre axis. The rodlets are themselves striated, giving rise to a concentric lamellar appearance of the segments (Nageotte, 1910, 1911).

In sharp contrast to this multitude of complicated structures seen in fixed preparations is the appearance of the sheath in fresh, teased fibres. Except for nodal discontinuities and occasional evidence of incisures in stretched fibres, such sheaths present no microscopic heterogeneities in visible light (de Renyi, 1929*a*), in ultraviolet light (Massazza, 1928), under ultramicroscopic conditions (Ettisch & Jochims, 1926; Auerbach, 1929), or in polarized light (see Schmidt, 1936). It has long been obvious that the wealth of complicated structures demonstrable in the sheath by the various cytological methods and the inevitable disagreement among cytologists as to which of these various pictures best represents the structure of the sheath in the fresh fibre are due to the extreme sensitivity of the sheath colloid to chemical and physical manipulation. Indeed, the problem of the organization of the nerve sheath is one of the most striking illustrations of the operation of the thanatological principle in the field of morphology.

Perhaps there can be no escape from certain aspects of this indeterminacy, considered in the strictest sense. Nevertheless, it is possible to apply to the problem certain optical methods which have little effect upon the ultrastructural metastability of the sheath, and it is the purpose of this review to show how the application of these methods has so far aided in the unravelling of these difficult problems.

II. THE ULTRASTRUCTURE OF THE MYELIN SHEATHS OF VERTEBRATE NERVE FIBRES

The chemical constituents of the myelin sheath, giving rise to the polarization optical and X-ray diffraction effects to be described in this section, are the lipoids and the proteins.

The lipoids may be classified under three general headings: phosphatids (lecithin, cephalin, and sphingomyelin), cerebrosides (cerebron, kersasin, nervon, and oxynervon), and sterols (chiefly cholesterol and its esters). For details of the chemistry of these compounds see Thierfelder & Klenk (1930), Bull (1937) and Page (1937). The asymmetries of these molecules are of importance in determining their strong tendency for orientation.

In terms of modern protein chemistry little can be said to be known definitely about the protein of the myelin sheath. On the basis of solubility and digestibility data Ewald & Kühne (1877) and Kühne & Chittenden (1890) classified this protein with the keratins, giving it the name "neurokeratin". As a result of a comparison of the relative amounts of certain constituent amino acids of neurokeratin and the

more typical keratins, the correctness of this classification has been called into question (Block, 1937). Because of the necessity of performing the analyses on large quantities of material, such as brain, it is impossible to be sure that the protein which is isolated and characterized is actually that of the myelin sheath rather than that of some other tissue component. Moreover, the intimate union of protein and lipid in the sheath requires the use of lipid solvents for separation, and this makes a determination of the normal properties of the protein extremely difficult.

(1) *Polarized light studies*

For a full historical account of this work up to 1936 and for the details of the optical methods the reader is referred to the books of W. J. Schmidt (1924*a*, 1937). For the convenience of those unacquainted with the theory and practice of the polarization optical method we include an appendix (p. 46) intended to explain in an elementary way the terms used in the present review.

The myelin sheath of a fresh fibre behaves optically as though it were composed of positive uniaxial elements with optic axes directed radially. This description is in agreement with observations made by viewing the fibres laterally and in cross-sections prepared by the freezing method. With respect to microscopic appearance and optical properties the myelin sheath shows close resemblance to the tubular myelin forms produced by the action of water on the lipoids extractable from nerve, either in a mixture or as single pure components (Göthlin, 1913; Cristini, 1928; Schmitt & Bear, 1937; and many others). That the optical properties of the sheath are determined predominantly by its lipoids is shown by the fact that the application of alcohol promptly reduces the birefringence markedly in magnitude and actually reverses its sign (Ambronn, 1890). It has been shown (Mezzino, 1931; Schmidt, 1936; Chinn & Schmitt, 1937) that this reversal of birefringence is due to the presence in the sheath of protein components which contribute negative uniaxial birefringence, the optic axis at any point being directed radially. Immersion experiments have demonstrated, moreover, that this protein birefringence is largely form birefringence, and, since its sign is negative, it may be concluded that the protein particles are anisodiametric and are oriented with their long dimensions disposed in planes parallel with the surface of the sheath.

The microscopic appearance of a lipid-extracted sheath depends largely upon the manner in which the preparation has been made. Usually the protein residue (neurokeratin) takes on the appearance of a mesh or network. Nageotte (1910, 1911) pointed out that under certain conditions there is a marked tendency for the protein to separate out in concentric lamellae or leaflets, a fact readily understandable from the optical analysis, which shows that the protein particles are oriented in a similar manner in the sheaths of fresh fibres.

The conditions under which neurokeratin networks are produced in the myelin sheath under the influence of lipid solvents have been analysed with the aid of the polarizing microscope recently by Schmidt (1936). He finds that in weak alcohol (30–50%) the lipid separates from the unified lipid-protein complex as droplets or spherites which show positive polarization crosses. The spherites, imbedded in

the protein meshwork, then begin to dissolve, the protein being simultaneously desolvated by the alcohol to give rise to the typical neurokeratin network. The stronger the alcohol the smaller the spherites, solution occurring before they can be built up to large size by addition of lipid molecules extracted from the sheath. With absolute alcohol the birefringence of the lipid disappears "mit einem Schlag" and the sheath shows a punctation which marks the regions from which lipid was extracted. The coarseness or fineness of the protein network will depend on whether the lipid is extracted as large spherites or as minute aggregates. Obviously, experiments such as those of Stübel (1912), in which changes in neurokeratin configuration are correlated with nerve activity, are of doubtful significance.

(2) *X-ray diffraction analysis*

Since the analysis is made on whole nerves, without the necessity of preparing individual fibres, the experimental conditions may be considered less abnormal than those of other methods, which invariably do require teasing out the fibres. Moreover, the amount of radiation sufficient to produce certain characteristic patterns appears to have little effect either upon the irritability or on the action potential of the nerve.

In a recent review (Schmitt, 1936) an account is given of the earlier work in this field. The present discussion will be based almost entirely on the papers by Schmitt *et al.* (1935, 1939).

The patterns obtained from a wide variety of fresh vertebrate myelinated nerves (amphibian and mammalian, peripheral and central) are all essentially alike. They are, moreover, to be ascribed entirely to the myelin sheath (other components, even if present in appreciable quantities, are not ordinarily detected except after drying the nerve).

The various diffractions obtained from fresh nerves, considered in the light of the optical evidence cited above, have been interpreted as indicating that the myelin sheath is constructed in the following manner. The basic pattern is one of cylindrical layers wrapped concentrically about the axis cylinder and containing lipid and protein molecules. Each unit layer has a thickness in a radial direction of approximately 171 Å. and is composed of two bimolecular leaflets of lipid bounded on either side by a layer of protein. The most probable configuration of such a system would be that in which polar groups of the constituent layers are in apposition to form predominantly aqueous phases. Alternating with these are the predominantly hydrocarbon phases containing the paraffin chains of the lipid molecules.

The radial organization has been deduced from the equatorial diffractions obtained from frog sciatic nerves and bull frog motor roots. These consist of spots representing orders of a fundamental spacing of 171 Å. This structure period appears to be unique for nerve, since in general pure lipoids or mixtures of lipoids as found in nerve give spacings to be expected of double layers only. This tendency of the lipoids to form bimolecular layers is commonly observed with long chain compounds of this nature. In the case of nerve it must be supposed that the inclusion of the protein results in the construction of the more complex unit. The particular

organization suggested for the nerve sheath rests largely on the facts that the nerve fundamental spacing is more than twice the average lipid period, and that alternations can be observed in the intensities of the spots assigned to the various orders of the nerve period.

In contrast to the definite, almost crystalline regularity of the sheath structure in the radial direction is the random distribution of the lipid molecules in the tangential direction, i.e. within the concentric layers. The hydrocarbon chains of the lipid molecules are distributed in a manner similar to that of liquids. The diffuse meridional diffractions at 4.7 and 9.4 Å. in the nerve pattern correspond to this aspect of the structure. These diffractions are not only of the proper magnitude but their meridional accentuation is also to be expected from this hypothesis. Moreover, the polarized light analysis strongly suggests this lack of well defined tangential organization, for the single optic axis at any point in the sheath is radially disposed.

Consideration of the semi-fluid consistency of the myelin sheath, as well as the organization outlined above, suggests description of the structure of the sheath (and the similar myelin forms) as being of smectic mixed fluid-crystalline character.

While it might be supposed that the layers containing predominantly the hydrocarbon chains are quite free from water, this is probably far from the case. Göthlin (1913) showed that the unsaturation of the hydrocarbon chains of these molecules is essential for the formation of myelin forms, presumably resulting in increased hydrophylic properties of the chains. Göthlin, and also Schmidt (1924*b*) supposed that a definite and limited range of water concentration in such structures is necessary, the lower limit corresponding to the amount needed to provide lubrication of the lipid molecules, permitting them to move with respect to one another, and the upper limit being determined by the amount of separation of the molecules which is possible without reducing their powerful mutual orientation effects too greatly. Indeed, consideration of the short-spacing diffractions of nerve as well as the available analytical data concerning the chemical composition of the sheath suggests that, while the lipid molecules must be considered fairly close packed, this does not mean that the hydrocarbon phases of the sheath are necessarily strictly non-porous. Actually the hydrocarbon chains probably occupy less than half the area available in the planes passed tangentially through the predominantly hydrocarbon phases of the concentric layers. Consideration of questions such as these is of importance in connexion with the permeability of the sheath to ions, metabolites, narcotics, etc.

In soft complex structures such as these, conclusions derived from optical and X-ray diffraction studies will represent only an average state of affairs. This makes it difficult to determine the structure with any great degree of precision. This is particularly true with regard to the details of the structure in the neighbourhood of the protein layers. It can be estimated, however, that in every 171 Å. period radially in the sheath, approximately 30 Å. of thickness are occupied by protein. This would seem to suggest that the protein is present as a single or double layer of particles, or perhaps even as thin sheets, such as those described by Wrinch (1937). It is an

interesting fact that the structure of the myelin sheath appears to be quite similar to the artificially constructed multilayered films of stearate and protein, described by Langmuir *et al.* (1937). Further investigation of the role of protein in determining sheath ultrastructure is highly desirable.

Certain quantitative considerations of sheath birefringence detailed below show that the variation of the relative proportion of lipid and protein in the axon sheath constitutes the chief sort of difference found between sheath structures of various types of axon. So far the X-ray diffraction method of structure analysis has been of value chiefly in the case of well myelinated vertebrate fibres; significant diffractions have not been obtained from fresh invertebrate nerves, presumably because of high water and low lipid content (see the Appendix).

III. THE ULTRASTRUCTURE OF THE AXON SHEATHS OF INVERTEBRATE FIBRES

As a class invertebrate nerves are generally regarded as unmyelinated, the axon being thought of as essentially naked except for a variable amount of closely adhering connective tissue. To be sure, certain invertebrate fibres have long been known to possess well myelinated sheaths (giant fibres of certain annelids and arthropods (Friedländer, 1889) and many fibres of prawns and shrimps (Retzius, 1890)). Nevertheless, from the histological point of view the great majority of invertebrate fibres do not appear to possess well-defined sheaths showing the histochemical reactions typical of myelin-containing structures. It is also true that the optical properties of nerves, composed on the one hand of myelinated and on the other of the so-called unmyelinated fibres, are very different. The latter type of nerve exhibits a birefringence which has the same sign as that typical of protein fibres (hair, tendon, muscle), i.e. positive with respect to length, while the heavily myelinated nerves show double refraction of opposite sign. Ambronn (1890), recognizing that gradations between these two extremes existed, and also that the characteristic birefringence of the well myelinated nerves gives way to the typical protein birefringence upon removal of the myelin or destruction of its orientation, early suggested that the actual birefringence observed with a given nerve is the resultant effect of two opposing factors tending to shift the sign of double refraction in opposite directions.

Göthlin (1913) considerably extended this view. It had been a practice among histologists to immerse tissues in media, such as glycerine or sugar solutions, which because of their high refractive index increase the transparency of the tissue. Göthlin found that when typical invertebrate nerves are so treated the birefringence undergoes a reversal of sign to that characteristic of the more highly myelinated nerves. This phenomenon, called by him the *metatropic* reaction (from the Greek *metatropos*, to reverse), could be prevented by preliminary treatment of the nerves with lipid solvents, hence was clearly a test for the presence of lipid in the nerves. Using it as such, Göthlin classified a large number of nerves from representatives of various phyla into essentially three groups: myelotropic, those normally possessing optical properties typical of lipoids; metatropic, those requiring immersion in the

glycerine for the detection of the myelin; and proteotropic, those which under no condition could be demonstrated to have sufficient lipid to overcome the optical characteristics of the background protein substances present in all nerves. His atropic and heterotropic groups are omitted here, since they either include doubtful cases or add no essential considerations.

It should be noted that Göthlin, while stating that the reversal of birefringence occurs at the periphery of the fibres, and describing the fibres as "blassrandig" and "dunkelrandig", did not localize the metatropic effect to any specific fibre structure. He referred to the lipoidal material as forming a cementing substance between the fibres. With regard to the mechanism of the reaction he was led by experiments on the chemistry of myelin forms, and on the birefringence of various individual lipoids when under the influence of mechanical stresses, to conclude that the phenomenon is an artifact introduced by tensions resulting from dehydration shrinkages of the tissue under the influence of the highly hypertonic solutions employed, and to place undue emphasis on the importance of cholesterol or its esters among the lipoids involved.

While from one point of view Göthlin's classification provides a valuable ordering of nerves with respect to lipid content, as Ambrohn suggested was possible, on the other hand his conception of the metatropic reaction delayed full realization of the significance of the similarities between the myelotropic and metatropic nerves. As long as it is supposed that the metatropic effects are dehydration artifacts and not related to any well-defined component of nerve or fibre structure, it is clear that any similarity in double refraction between, say, a myelinated vertebrate nerve and a glycerine-treated metatropic invertebrate nerve will be regarded as showing only that both have the common property of possessing myelin, not that they have similar myelin-containing axon sheaths. Since this latter point is now known to be true, as is shown below, it is obvious that Göthlin's conception of the metatropic reaction must have been incorrect or at least incomplete.

If we go back to Ambrohn's suggestion and regard various types of nerves as representing cases in which the lipid birefringence has had varying degrees of success in overcoming the protein contributions, we are led to inquire more closely into the nature of the principal protein structures of nerve. Two of these, axis cylinders of the nerve fibres and connective tissue strands, have long been recognized, morphologically, histochemically, and from the standpoint of polarization optics, but a third has been somewhat elusive. Though since 1877, when Ewald & Kühne first described neurokeratin, much has been written about the morphology and chemistry of this protein substance of the vertebrate fibre's myelin sheath, it was not until 1931 that Mezzino pointed out that the birefringence of the sheath protein was opposite in sign to that of the lipid. Finally, Schmidt (1936) described the optical characteristics of the sheath protein in greater detail (see the section above).

Kühne & Chittenden's (1890) suggestion of "neurochitin" as the substance of crustacean nerve corresponding to the neurokeratin of vertebrate nerve would seem to imply the existence of a sheath about the crustacean fibre similar to the myelin sheath. Attempts to demonstrate such a structure histologically have been largely

unsatisfactory; for example, compare the views of Lullies (1934) and Young (1935). Recently (Bear & Schmitt, 1937), application of the polarization optics, in conjunction with a reinvestigation of the nature of Göthlin's metatropic reaction, has led to detection of this invertebrate fibre component. The essential feature of these recent studies was the determination of the minimal conditions for reversal, i.e. the lowest concentrations of solutions of metatropic reagents compatible with reversing power. When reversal is accomplished under these conditions, dehydration distortion of the individual fibres of the nerve is reduced and it is possible to see that the part of the fibre which shows the characteristic lipid sign of birefringence is located close to the axis cylinder and inside the surrounding connective tissue layers, i.e. in a position similar to that of the myelin sheath of myelotropic fibres. To give Göthlin full credit, however, it should be stated that in one case (crayfish nerve) and using one metatropic reagent (glycerine) he performed a similar experiment and observed that the reversal occurred between or at the periphery of the fibres and did not involve connective tissue surrounding the nerve as a whole. Apparently he did not observe closely enough to see that the reversal did not occur throughout the interfibrillary regions and so gained the erroneous impression of a lipoidal cementing substance between the fibres.

Comparison of various properties of the solutions which will just bring about the metatropic reaction disclosed another important fact: there is good correlation between refractive index and reversing power (reversal in sign of birefringence occurring at an index of about 1.35 for lobster and *Limulus* nerve) and very little relation between osmotic pressure or fibre dehydration and the optical changes. Thus, at least under the more nearly normal minimal conditions for reversal, it is clear that Göthlin's view of the metatropic reaction is not correct. Birefringent protein tissues in general owe much of their double refraction to form birefringence, which is lowered by increase in refractive index of the solution surrounding the protein particles. In view of this fact the most reasonable explanation of the metatropic effect would be that application of solutions of high refractive index to a nerve reduces the protein contributions, from axis cylinder and connective tissue, as well as from sheath protein, allowing the lipoids, *normally present in oriented condition*, though in low concentration, to become evident. The lipid double refraction is largely of intrinsic or crystalline character, hence not dependent on the refractive index of the medium in which it is dispersed. Chinn & Schmitt (1937), by means of the immersion technique applied to frozen sections of invertebrate and vertebrate nerve, as well as to fixed preparations, have examined this conception of sheath structure in detail. The sheaths of both types were found to be similar with respect to the orientation of optic axes and to the type and sign of birefringence of the lipid and protein components.

Much of the difficulty encountered in attempting to localize the lipid-containing sheaths of typical invertebrate nerve results from the extreme thinness of these sheaths. While the thickness of the myelin sheath of vertebrate fibres is about 23 % of the axis cylinder diameter, the comparable figure for invertebrate fibres is of the order of a very few per cent.

In cross-section the typical histological picture of invertebrate nerve is that of axons surrounded by many collagenous strands with accompanying cell nuclei. Certain nuclei can be observed very close to the axis cylinders, even indenting these in some cases (Young, 1936*a, b*). Since there is insufficient lipid to produce very marked contrast between the connective tissue and the metatropic sheath in osmic acid preparations, it was difficult to obtain clear-cut histochemical evidence for a difference between the connective tissue layers and any more specific sheath layer, and the closeness of approach of the nuclei next to the axis cylinder made it difficult to correlate the histological and polarization optical pictures.

It was suggested to the authors by J. Z. Young that the giant fibres supplying the squid mantle muscles might, because of their large size, furnish good material for the solution of these problems. The subsequent demonstration (Bear *et al.* 1937) of the presence of a metatropic sheath about these axons not only added evidence from an additional phylum regarding the widespread occurrence of such structures about nerve fibres, but in this case it was possible to establish that the nuclei indenting the axon belong to a thin layer of cells located between the metatropic sheath and the axon. These cells, therefore, are to be regarded not as connective tissue but perhaps as analogous if not homologous to the Schwann cells of the vertebrate fibres. Retzius (1890) described a similar inverted relation between the medullary sheaths and associated cells of prawn and shrimp fibres. It will be of interest to determine how widespread this inverted relationship is among invertebrate nerves.

The squid giant fibres investigated are unusual in that they originate from the fusion of the processes of many small neurons. Since each large fibre can be shown to function physiologically as a single unit, despite its multiple origin, it may be significant that the metatropic sheath surrounds the entire axon, i.e. encloses a single physiological unit.

The historical approach to the solution of the invertebrate fibre sheath problem was adopted in this review since it presents in clearest fashion the difficulties encountered. The criticism of Göthlin's interpretation of his metatropic reaction is intended to be in no sense disparaging to the contributions of this pioneer, who worked at a time when the conception of form birefringence was just beginning to come into general use (Ambronn, 1910; O. Wiener, 1912). At any rate, even to-day the investigator of such problems finds in Göthlin's classification of nerves a valuable catalogue of useful materials. While at present the detailed analysis of the sheath and axon relations has been carried out for only a few cases, it may be expected that Göthlin's classification, combined with the modern viewpoint, will point the way in which the final results are to be sought.

IV. QUANTITATIVE CONSIDERATIONS OF SHEATH BIREFRINGENCE

If fine structure analysis is to lead eventually to an appraisal of the role of the sheath in nerve function, quantitative data must be obtained and these correlated with certain characteristics of the fibre, such as fibre diameter, sheath thickness,

etc., the latter, in turn being capable of correlation with physiological properties. Eventually, it may be hoped that the optical and electrical data may be obtained simultaneously from single teased fibres.

(1) *The myelin sheaths of vertebrate fibres*

The birefringence of the myelin sheath cannot be measured in the manner commonly used for other tissues, namely by dividing the observed retardation (measured with a suitable compensator) by the thickness of the object (diameter of the fibre). Since the optic axis at any point in the sheath is radial, the effective thickness is no obvious function of fibre or sheath dimensions. To make quantitative measurements possible the following expression was derived (Bear & Schmitt, 1936) relating the birefringence of the sheath ($n_a - n_0$) to the maximum retardation, Γ_{\max} , and to the diameters of the axis cylinder, d_2 , and of the fibre, d_1 :

$$n_a - n_0 = \frac{3 \Gamma_{\max}}{(d_1 + 2d_2) \cos^{-1} \left\{ \frac{(d_1 + 2d_2)}{3d_1} \right\}}.$$

Applying this formula, the birefringence of *A* fibres (10–20 μ .) of frog sciatics was found to be 0.011 (Schmitt & Bear, 1937). The contribution of the lipid component is even higher because of the opposing optical effect of the protein component. That this value indicates a high degree of orientation of the lipid is shown by comparison with similar substances in the pure condition: ammonium oleate crystals, 0.023; lecithin oriented by tension, 0.017; built-up films of barium stearate, 0.06 (Blodgett & Langmuir, 1937).

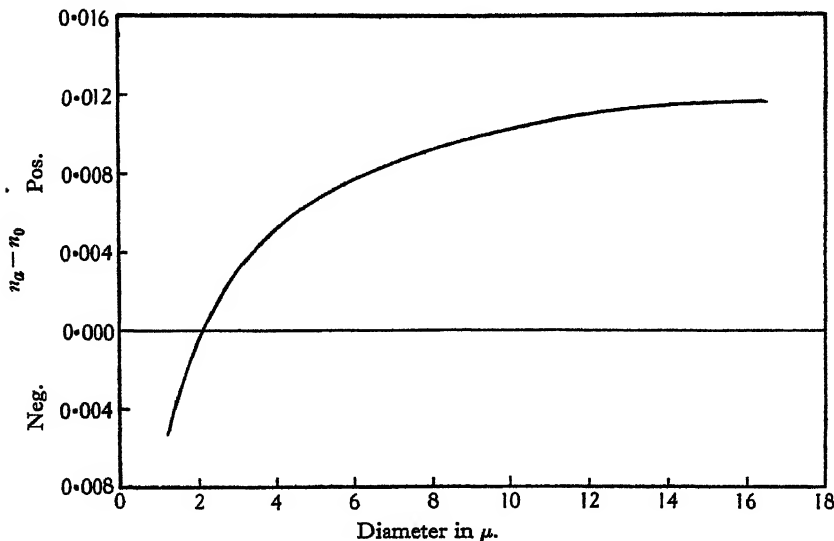


Fig. 1. The relation between birefringence ($n_a - n_0$) of sheath elements and fibre diameter in frog sciatic nerve fibres.

Fig. 1 shows the relation between birefringence of sheath elements and fibre diameter. The sign of the birefringence is referred to the direction of the optic axis

(radial); lipid components will therefore produce positivity, protein components negativity. The curve demonstrates that the chemical composition and molecular architecture, like the electrical properties (Blair & Erlanger, 1933), vary in a continuous manner throughout the range of fibre sizes. It is interesting that the transition from lipid predominance (optically indicated) to protein predominance occurs at a fibre size of about 2μ . This is precisely the point which Duncan (1934), on the basis of histological methods, designates as the dividing line, above which all fibres are "myelinated" and below which they are "unmyelinated". It is uncertain whether this curve is typical of all myelinated fibres; measurements on turtle vagus fibres show general agreement, but there is some indication that sheaths of certain small fibres in the central nervous system may be more birefringent than would be indicated by the curve. A detailed study of the birefringence of a wide variety of fibre types might well bring to light information of value not only for the fine structure of the fibres but for their physiological properties as well.

(2) *The axon sheaths of invertebrate fibres.*

No quantitative data are available concerning the manifestly myelotropic (highly myelinated) invertebrate fibres such as those of shrimps, the giant fibres of the earthworm, etc. Because of the thinness of the more typical metatropic invertebrate sheaths application of the formula for the calculation of the birefringence of sheath elements has little meaning in these cases. Rough comparisons of sheaths of different fibres can be made, however, by determining the minimal refractive index required for immersion reversal of sign of birefringence. Since the optical characteristics of the lipid and protein are probably similar in all fibres, the index of refraction required for reversal is a measure of the relative concentrations of oriented lipid and protein in the sheath. Comparisons of this sort (Bear & Schmitt, 1937; Bear *et al.* 1937) have indicated that fibres from different animals (lobster, crayfish, *Limulus* and squid) as well as of different sizes (small and giant fibres of squid mantle nerves) all fall within a limited range of relative lipid and protein content.

(3) *Physical factors underlying sheath structure*

The curve of Fig. 1 expresses in a quantitative manner the fact long known to histologists that the large vertebrate fibres are well myelinated while the small fibres are poorly myelinated or are "unmyelinated". Obviously a physical explanation of this fact presupposes an understanding of the molecular structure underlying the sheaths of large and small fibres. While admittedly the optical and X-ray analyses are still far from complete, a tentative suggestion has been offered to account for the observed facts (Schmitt & Bear, 1937). The well-developed myelin sheath may be regarded as a type of smectic structure in which concentric sheets of protein are interspersed between layers of lipids so as to form structures which are repeated periodically in a radial direction and which have on the average an unusually large periodicity (171 \AA). It is inviting to suppose that this tendency to form large structures results in a physical restriction of the amount of lipid that can be incorporated in sheaths of great curvature and still be compatible with physical

stability. There are grounds for supposing that the fibre diameter may be a more decisive factor than qualitative embryological and genetic differences between small and large fibres. Duncan (1934) found, for example, that all the fibres of the sensory roots of the cow are larger than the corresponding fibres in certain other smaller mammals, such as the rat. Corresponding with this increased fibre size he finds that even the smallest fibres are myelinated, thus suggesting that the diameter is the controlling factor determining myelination. Duncan cites many lines of evidence in support of this conclusion, and among these is the frequently observed fact that in embryonic development the larger fibres are the first to acquire myelin sheaths. On the assumption of aggregates 171 Å. long and 5 Å. thick, oriented with the long direction radial, Schmitt & Bear suggested that while radial packing of such long crystallites might be possible in large fibres, the greater curvature obtaining in small fibres might lead to strains which would render the system unstable. The more recent X-ray analysis, which pictures the smectic layers as being relatively fluid in the tangential direction, leaves little room for this specific explanation, though it would still seem probable that the essential idea of the restraint imposed upon construction of the large lipid-protein complex by the curvature of the smaller fibres is reasonable. The problem has similarities to the case of the increased vapour pressure of small droplets of a liquid and to the increased solubility of small particles of a solid. It is true that very small myelin forms can be made, whose birefringence is independent of diameter, but comparison of these with the sheaths of small fibres is rendered complicated by the presence of the protein component in the latter. On the other hand, the myelin forms tend to grow and increase their diameter, suggesting that even in these the greater curvature is a less stable configuration. It is to be hoped that with more knowledge of the properties of the sheath protein and perhaps with data bearing on the question as to whether the long spacing of 171 Å. is typical of the small as well as the large fibres, the physical interpretation of the relation between myelination and fibre curvature may become clearer.

(4) *Nerve function in relation to sheath ultrastructure*

It has been shown above that a wide variety of types of nerve fibres possess sheaths of more or less characteristic and similar chemical and optical properties. This fact immediately raises the question as to how important this sheath may be for physiological function. While a direct demonstration of chemical or physical changes occurring in the sheath during excitation or conduction would furnish the most conclusive answer to this problem, in the absence of such information it is of interest to attempt indirect examination as to a possible relation of sheath structure to function by making certain comparisons. The particular characteristic of physiological function chosen for these comparisons is that of conduction velocity, selected because it is a commonly measured property of nervous activity which appears most obviously to be influenced by sheath structure. Since the sign and magnitude of sheath birefringence are convenient structural measures, chiefly of the relative amounts of protein and lipid present, the correlation to be pointed out is that between sheath birefringence and the velocity of conduction of various fibre types.

This sort of indirect approach to the relation of a fibre component to function is comparable to that frequently used in attempts to discover the connexion between conduction velocity and fibre dimensions. For example, in the case of vertebrate fibres the velocity has been said to vary as the first, second, or intermediate power of the diameter (see Erlanger & Gasser, 1937). In what follows it is convenient to contrast the range of fibre types over which generalizations regarding the dependence of conduction velocity on diameter and sheath structure apply. This attempt is not to be construed, however, as suggesting that either factor is to be discarded in favour of the other. Indeed, the evidence as presented below shows that both are operative and are rather to be regarded as independent variables with different degrees of importance in affecting conduction velocity.

The facts of interest in the present connexion are given in Table I, in which fibres are classified according to sheath birefringence under three categories: metatropic, moderately myelotropic, and strongly myelotropic. It will be obvious that

Table I. *Relation of conduction velocity to fibre diameter and sheath ultrastructure*

Sheath type	Nerve	Fibre diameter μ .	Approximate conduction velocity m.p.s.	Reference
Metatropic	Frog sciatic nerve	ca. 2	0.4-0.5	Erlanger & Gasser (1937)
	Crab leg nerve	4	0.1-0.5	Lullies (1934)
	Crab leg nerve	4-8	1-2	Lullies (1934)
	Crab leg nerve	10-20	2-4	Lullies (1934)
	Crayfish claw nerve	ca. 10-20	3-4	du Buy & Coppée (1936)
	Squid mantle nerve	10-20	3-6	Young (personal communication)
	Squid mantle nerve	400-600	15-20	Young (personal communication)
Moderately myelotropic	Frog sciatic	3-8	ca. 4	Erlanger & Gasser (1937)
	Giant fibres, earth-worm ventral cord	60-80	17-25	Eccles <i>et al.</i> (1933)
Strongly myelotropic	Frog sciatic	11	17	Erlanger & Gasser (1937)
	Frog sciatic	19	42	Erlanger & Gasser (1937)

within a group of fibres possessing similar sheaths, the conduction velocity is a function of fibre diameter, large diameters favouring fast impulse propagation. On the other hand, between groups of fibres whose sheaths are quite different fast conduction is favoured by incorporation within the sheath of increasing relative amounts of lipid. On the whole the velocity of conduction is more greatly affected by the sheath structure than by diameter. It is particularly striking that to attain velocities approaching those of strongly myelotropic fibres, the moderately myelotropic ones must have diameters as large as 60-80 μ ., while metatropic fibres are forced to the enormous size of 400-600 μ .

While the correlation between sheath ultrastructure or composition and conduction velocity appears to be fairly striking, qualitatively at least, it is difficult to

make it quantitative, as much because of lack of physiological information as because of insufficient optical and chemical data. It is, therefore, not entirely certain that the connexion between ultrastructure and impulse propagation may not be only apparent, the true factors influencing conduction velocity being other variables which may happen to parallel somewhat the ultrastructural variations. For example, it has been stated that the relative thickness of the myelin sheath furnishes a clue to the conduction velocities of various fibre types (Bishop & Heinbecker, 1930; Lullies, 1934). This possibility is particularly apparent when typical vertebrate and invertebrate fibres are compared, the relative sheath thicknesses of the latter being much less than those of the former. However, it must be noted that the sheaths of these fibres differ as much in chemical composition as they do in thickness, a fact which cannot be neglected in considering the relation of such factors to function.

The frog sciatic nerve offers as wide a variety of fibres, when classified according to sheath ultrastructure, as any single nerve or group of nerves can be expected to show (see Fig. 1 and Table I). According to Donaldson & Hoke (1905) the thickness of the sheath of such fibres bears an essentially constant relation to axis cylinder or total fibre diameter. Actually, Schmitt & Bear (1937) found that the relative sheath thickness in such fibres shows a tendency to increase with decreasing fibre diameter. In any event the variations in thickness are not as definite as those existing between typical vertebrate and invertebrate fibres, hence it would appear that the success attained in Table I in ordering fibres according to sheath ultrastructure, without regard to sheath thickness, would argue in favour of the greater importance of intrinsic structure and composition.

Another factor that has been pointed out as paralleling myelination and conduction velocity is the internodal length of medullated fibres (Lillie, 1925; Gerard, 1931; see also Erlanger & Blair, 1934). Marshall & Gerard (1933), working with bull frog sciatic nerves found that in the case of fibres larger than 8μ . in diameter (hence strongly myelotropic and possessing sheaths of relatively constant structure) the conduction velocity seems to be related to diameter more simply than to internodal length. Whether this would permit exclusion of the nodes as being of significance compared to sheath ultrastructure and diameter in determining relative velocities of widely different fibre types is, of course, problematical at the present time.

Whatever may be the relative significance of these various factors in determining conduction velocity, it is worth emphasis that if the studies on the relation of velocity to fibre diameter are to have theoretical significance they must be carried out on groups of fibres whose sheath structures are comparable, the fibres differing chiefly with regard to diameter. Thus, attempts to include all fibres of frog sciatic nerves in a single law relating velocity of propagation to diameter alone, even if successful, would be of doubtful theoretical value.

V. CELLULAR SHEATHS AND MEMBRANES

(1) *Nerve cell sheaths*

Many unipolar nerve cells of vertebrates and invertebrates are enclosed in a closely applied investment containing numerous cells. In the crayfish and lobster this investment has been shown to be continuous with that of the axon (Ross, 1922; de Renyi, 1929*b*). It has also been claimed that the inner layer of the investment surrounding the vertebrate spinal ganglion cell is relatively poor in myelin and is continuous with the neurilemma (Kappers *et al.* 1936). The cells of this inner layer are presumably of embryological origin similar to that of the Schwann cells of the myelin sheath.

Chinn (1938), using the methods described above, showed that in these cases the nerve cell sheath is metatropic and can be distinguished from the surrounding connective tissue optically and chemically, the connective tissue being proteotropic and showing much greater swelling in alkali than the inner cell sheath. These neurons are therefore provided with sheaths containing oriented lipid and protein over their entire extent, except possibly at the nodes of the vertebrate sensory axons.

No such cellular sheaths were found to enclose the multipolar cells of the amphibian central nervous system. Chinn observed very weak birefringence typical of oriented lipid at the borders of these cells, but a number of considerations make the significance of these observations uncertain.

(2) *Cellular and nuclear membranes in general*

The great sensitivity of the optical technique involving the application of the metatropic reaction for the detection of oriented lipoids in a general background of oriented protein has suggested its use with cells other than those of nervous tissue. Though only few applications have been made these are of interest and are briefly listed as follows: The red cell envelope (Schmitt *et al.* 1936, 1938) has been shown to possess lipoids and proteins oriented in much the same fashion as in the typical invertebrate axon sheaths. Similar effects may be observed in the membranes of certain marine eggs and tissue cells (Runnström, 1928, 1929; unpublished experiments by the authors). The nuclear membrane of fresh nerve cells shows a clear polarization cross of sign typical of protein, no lipid being detectable after addition of media of high refractive index (Chinn, 1938). In other cells the nuclear membrane may be made to show a weak to strong polarization cross characteristic of lipid. These facts suggest the widespread occurrence at cellular and nuclear boundaries of structures composed of protein lamellae arranged in concentric layers, with variable amounts of oriented lipoids present within the protein matrix.

(3) *The plasma membrane*

The whole background of permeability and narcosis studies points to the fact that the plasma membrane is a complex of lipoids and proteins (see, for example, Höber, 1936). Recent work along a number of lines has been directed toward a more detailed knowledge of the actual structure of the physiologically important

membrane. These studies involve investigations concerning interfacial forces at cell and protoplasmic interfaces (Danielli & Harvey, 1935), impedance of the membrane to electric current flow (Fricke, 1933; Cole & Curtis, 1936), and models of membranes (Danielli, 1936). The point of view has been developed that the effective part of the membrane is an extremely thin layer of oriented lipoids and protein, at the most only a few molecular layers thick.

The case of the mammalian erythrocyte envelope is particularly interesting, for chemical data are at hand to show that the amount of lipid available in the entire cell is sufficient only for a close-packed surface film of this extreme thinness (Gorter & Grendel, 1925), and it is now known that most of the lipoids are indeed concentrated in the envelope (Erickson *et al.* 1938). While the polarization optics has furnished a direct demonstration of oriented lipid in the erythrocyte envelope (Schmitt *et al.* 1936, 1938), it has been pointed out that, because of inherent limitations of the microscope in localizing birefringent components within structures of dimensions approaching the limit of resolution, the optical method is unable to confirm or disprove the existence of *continuous* bimolecular layers of lipid within the envelope, though the data are in agreement with the conclusion that all of the cell's lipid is concentrated in this structure. In the case of the only cellular sheath to which the application of the X-ray diffraction method has been successful, namely, the myelin sheath of nerve fibres, it has been shown that the lipoids are in fact probably arranged as continuous oriented bimolecular layers of lipid, though in this case the sheath contains many such bimolecular layers. At present it is not possible, however, to pass from this case of a thick sheath to the optically similar thin membranes more frequently found about axons, cells and nuclei, and to suppose that the optically observable membranous structures are necessarily the physiologically important ones. This is particularly apparent in the case of the myelin sheaths of vertebrate fibres, for the capacity of the myelin sheath is not very different from that observed with other cells which are not nearly so strongly myelinated (Cole & Curtis, 1936). It is somewhat of a problem, however, to understand how such a thick structure, containing many bimolecular layers, could be without effect throughout its entire thickness on the electrical and chemical accessibility of the axon. In view of the indications given above of the apparent relation of nerve sheath ultrastructure to function, it is to be expected that the solution of problems such as these will be found to be of importance for a further understanding of membrane phenomena in general and of nerve excitation and conduction in particular.

VI. SUMMARY

1. In avoiding certain inherent indeterminacies in classical morphological methods and in obtaining further details regarding the microscopic and ultra-microscopic structure of nerve axon sheaths, the methods of polarization optics and X-ray diffraction are of great value. In the case of the myelin sheaths of vertebrate nerve fibres, for example, the optical and diffraction studies indicate the structure of the living fibre's sheath to be of smectic mixed fluid-crystalline nature.

The structure is, therefore, readily altered by chemical treatment to form the artifacts commonly observed in histological preparations.

2. A number of considerations suggest that the specific configuration of the lipid and protein components of the myelin sheath is as follows. The proteins occur as thin sheets wrapped concentrically about the axon, with two bimolecular layers of lipids interspersed between adjacent protein layers. While this means that in a radial direction within the cylindrical sheath there are alternate predominantly aqueous and predominantly hydrocarbon phases, the latter cannot be described as being entirely "non-aqueous".

3. Polarization optical studies show that, contrary to the general view, invertebrate nerve fibres quite widely possess, aside from connective tissue investments, thin sheaths which are essentially similar in ultrastructure to the well-defined myelin sheaths of vertebrate fibres. The demonstration of this fact involved a reinterpretation of the meaning of Göthlin's metatropic reaction, in which immersion of the fibre in media of high refractive index permits the (intrinsic) birefringence of lipoids present in the normal sheath in an oriented condition to become apparent by the reduction of the masking (form) double refraction of protein. Associated with the invertebrate metatropic axon sheaths are cells similar to the Schwann cells of vertebrate fibres.

4. Quantitative birefringence studies have disclosed that the axon sheaths of a wide variety of fibre types differ chiefly with respect to the relative amounts of oriented protein and lipid present. This difference is observed not only between typical invertebrate and vertebrate fibres, but also when the fibres of a single vertebrate nerve are compared. For example, the curve obtained when sheath birefringence of frog sciatic fibres is plotted against fibre diameter shows wide variations in the magnitude of double refraction, changing continuously from birefringence due preponderantly to lipoids, in the case of the larger fibres, to that which, in the smallest fibres, results primarily from proteins. The transition from lipid to protein predominance occurs at a fibre diameter of about 2μ ., agreeing well with the division between "medullated" and "non-medullated" fibres arrived at by histologists. It has been suggested that the low concentration of lipid in the sheaths of small fibres is related to physical factors opposing the introduction of the lipoids into cylindrical structures of high curvature.

5. Examination of available information with respect to the relation of the velocity of impulse propagation to certain fibre characteristics, such as diameter and sheath ultrastructure, indicates that in a wide variety of fibres conduction velocity is a function of both of these factors. Thus, if fibres from invertebrate and vertebrate sources are classified according to sheath composition and ultrastructure, it is found that, within a group having similar sheaths, fast conduction is favoured by large diameter, while between groups with different sheaths, heavy myelination results in faster propagation. Comparison of fibre velocities with diameter alone, without regard to degree of myelination, is apt to be confusing, a fact which should be borne in mind in attempting to relate conduction velocity to diameter in a nerve, such as the frog sciatic, which contains fibres with very different sheaths.

6. Several types of invertebrate and vertebrate unipolar ganglion cells have been observed to possess investments similar to the axon sheaths and continuous with the latter. The entire surface of these neurons, therefore, is provided with a characteristic lipoid-protein covering, except possibly at the nodes of the myelin sheaths of the vertebrate sensory axons. The limiting envelopes of certain other cells and nuclei have been shown to possess an ultrastructure similar in type to that of the axon sheath. Permeability studies on cells have indicated the importance of lipoids and proteins in determining the properties of the plasma membrane, but it cannot be concluded that the visible envelopes are identical with the membrane which determines the physiological properties, since electrical and chemical studies favour the view that this membrane is extremely thin. The parallelisms observed between nerve sheath ultrastructure and physiological function, however, suggest some relation of these to membrane phenomena, and it is particularly difficult to understand how a multilayered structure, such as the vertebrate axon's myelin sheath, could fail to influence the chemical and electrical accessibility of the axon.

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VIII. APPENDIX

Physical considerations regarding the application of polarization optical and X-ray diffraction methods

From the point of view of simple optical considerations, light may be regarded as an electromagnetic wave phenomenon with periodic variations in electric and magnetic field intensities occurring at right angles to the direction of propagation. If, in addition to being restricted to planes transverse to the propagation, the vectors describing the electric (or magnetic) field strengths at all points along a beam are further limited to a plane which includes the direction of propagation, the light is said to be *plane polarized*, the *plane of vibration* being the plane of the *electrical* vectors. Frequently one encounters more complex types of polarization. These are most simply considered by resolving them vectorially into two mutually perpendicular plane polarized components of equal or unequal amplitudes of vibration, the two being separated in time of maximum action at any point by a fraction of a period, written as θ in the discussion below.

In a vacuum the electromagnetic vibrations travel unchanged with the familiar velocity of 3×10^{10} cm./sec. Because of the electrical nature of matter, interactions occur between the light and the environment in the case of transmission through non-empty space. As a

result, the velocity decreases (refraction) and amplitude diminishes (absorption). For weakly absorbing materials (such as many tissues) only the velocity decrease is of importance in the visible range. The velocity change is usually expressed in magnitude by the *refractive index* of the material, this quantity being the ratio of the velocity of light in a vacuum to that in the medium under consideration.

If a material is isotropically constructed, its structure is, of course, the same in all directions from any point, hence the interactions between it and transmitted light will be the same no matter what the initial state of polarization of the light or the direction of transmission through the material. Thus we may say that such substances as gases and liquids (at rest), or unstrained amorphous solids (glasses), are characterized optically by a *single* index of refraction.

As an example of the case of transmission of light by *anisotropic* substances, consider a thin sheet of such material placed with its surfaces perpendicular to the direction of propagation of a beam of plane polarized light. The angle between the plane of vibration of the polarized light and some fixed direction in the sheet can still be varied, and if this is tried, one position will be found which allows the most rapid transmission and which leaves the initial state of polarization of the light unaffected. Any other orientation will have the following effect: immediately upon entering the sheet a component (of the initial plane polarized vibration) which is itself plane polarized, though oriented for fastest transmission, will speed ahead while the remainder, necessarily vibrating perpendicularly to the faster component, will lag behind by an amount determined by the properties of the material. Thus the anisotropic sheet acts upon the light in a manner which can be completely described in terms of a direction of vibration for faster (or slower) transmission and *two* velocities of transmission or indices of refraction. It is therefore said to be *doubly refracting* or *birefringent*.

Usually with biological objects the directions of polarization for faster and slower transmission coincide with directions of well-defined dimensions of the object, e.g. longitudinal and transverse directions of fibres. It is customary to define the *magnitude of birefringence* as being equal numerically to the difference between the two descriptive refractive indices. The convention with regard to sign is discussed below. Experimentally the two indices are rarely determined, only the difference between them being calculated in the following manner: If the incident polarized light of the above example is vibrating at 45° to the directions of polarization for fast and slow transmission, it can be shown that

$$n_1 - n_2 = \frac{\theta \lambda}{d} = \frac{\Gamma}{d},$$

where n_1 and n_2 are the two indices of refraction, θ the fraction of a period separating the two components as they emerge from the sheet, λ the wave-length of the light, and d the thickness of the sheet. The quantity $\theta \lambda \equiv \Gamma$ is called the *retardation*, since it is the distance that a point on the wave of the slow component is retarded behind the corresponding point on the wave of the fast component. Γ and d are the experimentally determined quantities, and since the retardation is proportional to the length of path travelled by the light (d), it follows that Γ/d , the birefringence, is independent of the thickness of the sheet, being an intrinsic property of its material and organization.

The example given above illustrates the results of optical observation on an anisotropic material using only one of the many possible directions of transmission, and as such it describes the considerations involved in studying an object from one view only, e.g. as with a muscle fibre viewed laterally. In general for anisotropic objects as many as three different refractive indices may be required to describe all the observations which can be made from the various possible directions. Instead of considering cases of this complexity, which are rarely encountered in biological materials, it is more useful to discuss the following simpler example of the extension of the optical considerations to three dimensions. Consider an object of cylindrical external form and suppose that corresponding to its external appearance

its ultrastructure is such that the axial direction is different from the transverse (radial) directions, all of the latter being indistinguishable. This is the case, for example, in fibres of muscle, tendon and hair. If we now examine rays transmitted through the cylinder in various directions we shall observe the following facts: Rays running parallel to the cylinder axis (perpendicular to a cross-section), hence with electric vibrations parallel to diameters of the cylinder, will be transmitted unchanged with respect to polarization, all polarizations being propagated with equal velocity. This results from the identical nature of the radially directed electrical fields of the material. On the other hand for transmission through the cylinder at right angles to its axis, the rays whose vibrations are parallel to the axis will encounter different electric fields than those vibrating parallel to diameters, hence these two polarizations will be transmitted with different velocities. In fact the refractive indices of the material for the longitudinal propagation with vibration paralleling cylinder diameters and for the transverse propagation with vibration again paralleling diameters will be the same, and can be designated as n_0 . Another refractive index n_a will be effective for the transverse transmission of longitudinal vibrations (parallel to the cylinder axis). Vibrations which are parallel with neither a diameter nor the axis will show a more complex behaviour, which can, however, be calculated from the values of n_0 , n_a , and the orientation of the polarization. It is, therefore, customary to say that the two refractive indices, n_0 and n_a , characterize the cylinder optically.

It will be observed that the longitudinal direction in the above example is unique both structurally and optically. Transmission along the axis is without double refraction, and this is the only direction of propagation for which this is true. Such a direction is called an *optic axis*, and the above cylinder, possessing only one such axis, is described as being *uniaxial*. Uniaxial symmetry is the type usually found in biological tissues, and when the single optic axis of a material is determined, one immediately knows the direction about which ultrastructure is isotropic and along which the construction is anisotropic.

As in the case of the examination of a birefringent object by transmission in a single direction (first example), the *magnitude* of the uniaxial anisotropy of the three dimensional case (second example) is expressed as the difference between the two refractive indices, n_a and n_0 . With respect to sign, however, the designations in the two examples may or may not agree. The words *positive* and *negative* are used in two senses to describe birefringent objects. In describing a *single* observation with *one* direction of transmission the object is said to show positive or negative birefringence *with respect to a distinguishing direction* (e.g. fibre length) according to whether the index of refraction for vibrations in this direction is greater or less, respectively, than the index for the other vibration perpendicular to it. The second type of description is used when the orientation of the optic axis is known, and then the material *as a whole* is described as being *positive* or *negative uniaxial* depending on whether the index for vibrations paralleling the optic axis (n_a) is greater or less, respectively, than that for vibrations perpendicular to the axis (n_0). The latter description is more fundamental, obviously, since it represents a complete qualitative description of the birefringent properties of the material.

With most filamentous structures (nerve axis cylinder, muscle, tendon, hair, chromosomes, etc.) the two ways of stating sign agree, since the conventional reference direction and the optic axis both coincide with the length of the fibre. In the case of the nerve axon sheath, however, the situation is apt to lead to confusion in the mind of the inexperienced reader. For example, while a well myelinated nerve fibre, viewed from the side, appears *negative* with respect to fibre length at the edges, because of the presence of the myelin sheath, actually the sheath substance at any point is *positive* uniaxial with radial orientation of the optic axis. The same is true of the myelin-poor sheaths of typical invertebrate axons or of lipid extracted myelin sheaths, except that the signs are reversed. In the text the more fundamental reference of sign to the radial optic axis is made, though the designation with respect to fibre length is found perhaps more frequently in the original literature.

It should be stressed, for critical consideration of the results of optical analysis, that

the conclusions which can be drawn with regard to ultrastructure are limited. All that can be said is that they determine directions in the material about which the physical stresses or molecular orientations are disposed in a manner such as to produce the observed optical effects. Magnitude of birefringence has little direct significance, except where materials of similar structure and possessing identical chemical constituents are to be compared. Even the sign of birefringence in itself means little, but it does frequently happen that the sign is a powerful aid to determination of chemical composition of the structures involved when regarded in the light of previous experience with simpler or more fully investigated materials. In general, fibrous structures of protein or carbohydrate nature are positive with respect to the fibre length, while lipoids and nucleoprotein are integrated into such structures so as to give the reverse or negative sign. Effects of various chemical agents in abolishing or changing the sign of birefringence also aid the identification of the type of substance involved in a given instance.

The limitations of the simpler optical methods are a result of the fact that the structural units are much smaller than the wave-length of the light employed, even though they may be large in the molecular or atomic sense. It is possible, however, by special methods to determine certain information regarding these units, and to do this it is necessary to consider the origin of birefringence in tissues. Causes of birefringence are frequently classified under three headings: (1) crystalline birefringence, such as results from the anisotropic fields produced by the regular array of molecules or atoms in typical individual crystals; (2) the true "photoelastic" birefringence, such as is found upon straining an isotropic solid; and (3) the so-called "form" birefringence, resulting when particles of *non-spherical* shape are preferentially oriented in a surrounding fluid of *different* refractive index. The second type of birefringence is probably of little importance in the soft semi-fluid structures comprising most biological tissues, and it is usually found that combinations of the first and third are sufficient to account for the observed optical properties. The relative contributions of the two types are readily ascertained by taking advantage of the fact that the form birefringence is markedly influenced in magnitude by the refractive index of the medium impregnating the tissue, being completely abolished when the particles and the medium surrounding them have the same index, while the crystalline birefringence remains constant during the immersion experiments, vanishing only when injurious agents, such as alkali, are added. The sign of the form birefringence gives evidence as to orientation of the long dimensions of the ultramicroscopic particles: *positive* uniaxial form birefringence signifies that the long dimensions of the particles are oriented so as to *parallel* the optic axis (as with parallel rodlets) and *negative* uniaxial form birefringence results from particles whose long dimensions are in planes *perpendicular* to the optic axis (as with parallel platelets). The immersion technique does suffer from certain of the criticisms frequently applied to histological methods, inasmuch as it usually requires fixation of the tissue. The justification of its use lies in the fact that, in general, the results lead to reasonable explanations of facts which can be determined on material in normal or nearly normal environments.

In order to go to molecular dimensions in studying tissue ultrastructure it is necessary to employ electromagnetic vibrations of wave-length smaller than the dimensions of the structural features to be observed, and here X-rays are useful. Since a number of discussions of this diffraction method are available in English (cf. Clark, 1932; Astbury, 1933; Sponsler, 1933) no details will be given here except those required to contrast the applicabilities of the optical and X-ray approaches to problems regarding axon sheaths.

In the first place the optical method offers a much more sensitive means of detecting anisotropic structures. For example, in nerves only the highly birefringent myelin sheaths of well myelinated fibres give good X-ray diffractions. The diffraction method has so far failed to be of very great value with the typical invertebrate axon sheaths, though these are readily investigated by means of the optical methods. The absence of diffraction by the invertebrate sheaths may be ascribable either to a deficiency of these structures with respect

to well defined and frequent repetitions of similar molecular configurations, which are required to produce good diffractions, or to lack of a sufficiently high concentration of diffracting material.

However, when diffraction evidence is obtainable from a given material, a single diffraction pattern can yield information not only as to orientation of the particles involved but also regarding the frequency with which a certain characteristic structure is repeated. Further, the distinctness of a spot or ring of the diffraction pattern is interpretable in terms of the manner in which the structure periodicity occurs, or in other words it indicates whether the repetition is as definite and as often repeated as in a crystal or is less distinct, as in a liquid. In general a single frequently repeated structural periodicity or *spacing* is shown by a series of diffraction rings or spots which are termed *orders* of a fundamental diffraction. Considerations based on the relative intensities of these orders furnish a certain amount of information regarding the *intra*-periodicity construction of the material. All of these types of evidence have been utilized in describing the nature of the myelin sheath organization. Though the optical method alone could only suggest in a general way the nature of this organization and is powerless to establish it in a detailed manner, yet the optical results are of great aid in guiding the analysis of the X-ray data. The combination of the two methods in the study of the axon sheath is one of the best examples of the value of the two methods when used complementarily.

One other distinction between the optical and X-ray diffraction methods is perhaps worth noting. When a ray of polarized light passes through a complex tissue, such as a nerve (composed of many myelin sheaths and axis cylinders, as well as connective tissue, etc.), the final state of polarization of the ray is the result of all of its experiences during the penetration and is not related in a simple way to the properties of any single component of the tissue. The X-ray diffraction pattern of the tissue, on the other hand, is the result of the superposition of the patterns for all the diffracting units present and separately considered. If, as in the case of the nerve myelin sheath, only one component of the tissue is strongly diffracting, the resulting pattern of the *total* tissue is essentially that of the *single* units composed of the highly diffracting material. Thus the diffraction pattern of a fresh vertebrate *nerve* may be considered as being very nearly that which would be obtained from an internodal segment of a single nerve *fibre*, the only differences being those slight ones introduced by lack of perfect parallelity of the fibres, by the curvature of the sheaths at the occasional nodes, and by possible differences in structure between very large and very small fibres. Though the optical method, therefore, requires extra manipulation in order to isolate single fibres, this is perhaps no serious disadvantage, since after the isolation the features of structure may be examined in smaller detail, e.g. the nodal structure can be examined, a possibility which has not been realized as yet with diffraction technique.

ADDENDUM

Since submission of this manuscript a paper by R. J. Pumphrey and J. Z. Young (*J. exp. Biol.* 15, 453, 1938) has appeared relating chiefly to the conduction velocities of *Sepia* and *Loligo* fibres. Working over a wide range of fibre diameters they find that the conduction velocities vary approximately as the square root of the axon diameters. In discussing their results in relation to the known facts concerning fibres from widely different forms, somewhat after the fashion of our section IV, 4 (p. 39), they are led to emphasize the importance of sheath *thickness* as well as fibre diameter in determining conduction velocity. This emphasis is based in part upon a misinterpretation of our own published data regarding sheath thicknesses of various fibre types. While not wishing to deny the possible importance of sheath thickness in determining physiological function we feel, as stated in the text (p. 41), that the information available at present seems to emphasize the importance of sheath *composition* and *ultrastructure* rather than that of sheath *thickness* as such.

PHASIC DEVELOPMENT OF PLANTS

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(Received 19 April 1938)

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I. INTRODUCTION

"VERNALIZATION" is one of the more controversial subjects of recent years, both among plant physiologists and agronomists; some investigators relegate it to a "mere modification of Gassner's chilling method", while others wish to include within the term the entire biology of plants.

In undertaking this review an attempt was made not so much to give an accurate account of the most important literature on the subject, as, by using a relatively limited number of papers, to present the facts and principles upon which the theory and practice of vernalization have been based. Consequently, it is not claimed that the bibliography appended is an exhaustive one or that the subject is dealt with in full detail. Further, the theory was elaborated in research with annual monocarpic plants; hence in speaking of plant development, it is intended to convey the sexual development or, as it is sometimes called, the "development from seed to seed", of these plants chiefly.

(1) *Discovery of phasic development*

In the complex investigation of "the physiological cause of the destruction of field crops" the study of the vegetative period in relation to environment has become particularly important in the extreme continental climate of the European and Asiatic plains of the U.S.S.R. The discovery of phasic development and consequent elaboration of the theory and method of vernalization are an outcome of

the studies undertaken by Lysenko in 1926-7 to investigate the exact cause of the failure to ear in the so-called winter plants when sown in spring.

Prefacing his analysis of the effect of thermal conditions on the duration of various developmental phases in cereals sown in the open on every tenth day throughout the year, Lysenko (1928) then postulated a *prima facie* distinction between growth and development, understanding by the latter a sequence of "biological phases" terminating in reproduction; the duration of each phase and hence the length of the entire vegetative period are governed, on the one hand, by the nature of the plant and, on the other, by those conditions under which the plant grows; in particular, it was established that, as distinct from other periods, that from appearance of seedlings to jointing was shortened with increasing temperature in some sowings, whereas in others it was prolonged or the jointing was even inhibited altogether. (The term "jointing" as used in this review is intended to imply elongation of the apical meristem, frequently referred to as shooting.)

It was observed, however, that if seedlings or even sprouting seeds were exposed to low temperatures for an adequate period, as in autumn sowings, the jointing stage occurred earlier under the subsequent high temperature; on the contrary, in plants sown by the end of winter or in the spring the time required to attain jointing was the longer the shorter was the period of exposure to low temperature before the temperature rose in the spring; finally, after still more advanced sowings, the plants failed to attain jointing and remained in the rosette stage.

The fact that winter crops require low temperature was indeed known and recorded in agronomy centuries ago, yet it remained for Gassner (1918) to demonstrate that if seeds of winter cereals were germinated at temperatures below 3-5° C. the resulting plants would, irrespective of the time of planting, ear in the first year, i.e. winter plants are endowed with a distinct requirement of low temperature at the early part of their growth. Gassner's conclusions were subjected to many tests, with conflicting results. Most investigators confirmed the causal effect of low temperature, but related the efficacy of the "chilling" method with the time of sowing or more precisely with day length subsequent to sowing.

Lysenko (1928), however, went further and concluded that "in the period from appearance of seedlings (and even from sowing) to jointing there are two 'consecutively connected' phases", each with a different relationship to thermal conditions. "The second of these consecutive phases which we term proper jointing is speeded up with an increase in temperature", and "the first, the progress of which leaves no morphological traces on the plant, on the contrary, is accelerated with a decrease" from a definite thermal maximum. Above this maximum this phase cannot commence and the plant will remain sterile. The first of the "consecutive" phases later became known as the stage of vernalization (after Lysenko) or the thermo-stage (after Vavilov) and is referred to in this review as the first phase.

Furthermore, as in these experiments the number of winter varieties, i.e. those failing to ear in the sowing year, increased with later sowings, it was concluded that any of the cereals may behave as a winter or spring form according to the time of sowing, and that one of the factors preventing earing is an after-sowing temperature

exceeding a critical maximum for the first phase. This maximum varied with varieties, and in this respect there is no clear demarcation between spring and winter plants, i.e. the principal difference between winter and spring plants vanishes and moreover failure to ear in the sowing year or delayed earing may, as will be shown later, be due to some other environmental factors. Hence this classification of plants is only relative, closely connected with time and place of sowing, as repeatedly reported by many investigators both before and after the announcement of Lysenko's principles.

As is known, neither the need for "winter rest", nor the specific length of the vegetative period stood the test as the criterion for this classification; nevertheless, the problem of winter and spring plants has been elucidated by Lysenko with results anticipated by Gassner (1918).

(2) *Vernalization*

In subsequent studies with cereals (Lysenko, 1929), slowly growing seeds were chilled (2 March) for a varying number of days and sown together with unchilled seeds on every second day, part in the field and part in pots in a greenhouse at a somewhat higher temperature. Wheat plants from unchilled seeds came into the jointing stage only after the first four sowings; those from chilled seeds attained jointing after all dates of sowing, but the percentage of jointing plants (invariably lower in the greenhouse) decreased with the later dates of sowing until 5 April.

Had all the chilled plants not attained jointing, when planted after 7 April, the concurrent interference of the seasonal day length might have been supposed. The absence of any effect of day length on the time of jointing was still more pronounced with barley; although jointing was progressively retarded in chilled plants of the March sowings, yet after later plantings the chilled plants came into jointing within the same number of days. In the time of jointing, therefore, only the effect of duration of chilling and of the rise of after-sowing temperature is evident; the latter retarded jointing until the chilling period was less than 38 days for wheat and 28 days for barley, i.e. a definite period of chilling is required to eliminate the retarding effect of after-sowing temperature. Actually, when the seeds of wheat and oats chilled for 50 days or more were planted in September, all the plants came into jointing or earing by November.

While investigating the effect of temperature on the length of the developmental phases in cotton, Lysenko (1928) concluded that high temperatures are not required for budding as such, but for those processes which later lead to the inception of floral organs; actually, seedlings kept for 15–20 days at 25–30° C. formed buds and flowered at 10–15° C., whereas plants grown invariably at 10–15° C. did not flower. On the assumption that the high temperature requirement for completion of the processes leading to flower formation in cotton can, irrespective of the age of plants, be satisfied before sowing, Lysenko in later experiments (1931) kept slightly sprouted seeds at a high temperature; again only the plants from treated seeds flowered at lower temperatures.

Lysenko, therefore, not only brought together the results of previous investiga-

tions, but by discriminating between growth and development, he deciphered the effect of temperature during the early stages, i.e. definite thermal conditions are required for a definite period to complete the first developmental phase. The method of germination in cold was thus converted into the method of vernalization, i.e. the subjecting of previously germinated and slowly growing seeds to a definite complex of environmental factors for a definite number of days. Herein lies the difference between the two methods. Subsequent experiments have not only confirmed the possibility of vernalizing various agricultural plants, but have also led, it is claimed, to the verification of Lysenko's hypothesis and to the subsequent announcement of the main principles of his theory.

II. THE FUNDAMENTAL PRINCIPLES OF LYSENKO'S THEORY

(1) *Growth and development*

From the theoretical and practical aspects the statement that "the development of a plant and its growth are not identical" is the keystone of Lysenko's theory. By growth Lysenko (1928, 1929, 1932*b*, 1932*e*, 1935*a*) understands the functions pertinent to changes in the size and weight of any organ of a plant, and by development a sequence of internal readjustments which the plant undergoes in ontogenesis, in its advance to sexual reproduction.

The rates of growth and of development in a plant are governed independently of each other by its environment, appearing thus as a result of the response of the plant to external conditions. As the conditions favourable for growth may, in some instances, be unfavourable or less favourable for the development of the same plant or vice versa, growth and development may be speeded up differently in a plant. This principle, excluding, perforce, any functional connexion between growth (vegetative development) and development (reproductive development) as advocated by Klebs (1913) and Maximov (1925), was substantiated in recent investigations (Murneek, 1937) where the photoperiodic inhibition of growth (curtailment of growth) was brought about independently of the photoperiodic induction of development (inception of reproduction) when the plants were kept in short days for a time longer than that required for the initiation of reproduction.

The rate of development is not only independent of the rate of growth, but need not necessarily be concurrent with or subsequent to growth; development may be initiated and progress as normally in a green plant as in a slowly growing seed; being independent of the size and age of a plant, the rate of development is governed by environment only, changes in which will affect the rate of development, but again similarly in a tillering plant and a slowly growing seed. These were the postulates for the method of inducing a part of development in slowly growing seed before sowing which has become known as the method of vernalization. This method of completing development will be referred to as *vernalization*, the duration of pre-sowing treatment as the *period of vernalization*, the plant subjected to the treatment as *vernalized*, and the time required from sowing to completion of development as the *vegetative period*. In these terms the period of development of vernalized

plants consists of the period of vernalization and the vegetative period, whereas the period of development of unvernized plants consists of the vegetative period only. /

Discrimination between growth and development is not new, as such ideas are traceable as early as Klebs (1913). The new and important aspect of Lysenko's conception is, firstly, the exclusion of the causal connexion in time between growth and development, as held by Klebs and his followers, and secondly, a new outlook on the interrelationship between the plant and its environment. Smith (1933) also emphasized the need to discriminate growth from development and concluded that "a discrimination must be made between the influence of the length of day on development and growth". This was ratified by Ljubimenko (1933, 1934) who also advocated the consideration of "the process of growth and the formative process as two physiologically different processes, although in normal development they usually proceed concurrently, as if interconnected"; in his experiments with a pea embryo grown in distilled water without cotyledons, and with lupin plants defoliated in various ways, growth was reduced in proportion to the decrease in the assimilative area, and yet the mutilated plants flowered at the same time as the intact control, thus showing that growth has only a quantitative aspect, i.e. it concerns the size and number of reproductive organs, but has no effect on their rate of development.

Further, there is no replacement of the vegetative phase by the reproductive; "the purely vegetative phase", in Ljubimenko's opinion, "exists only during embryogeny and the first phase following seed germination"; later, the reproductive development and growth proceed concurrently until the death of the plant. At first, such a discrimination between the time of commencement of growth and of development seems to diverge from Lysenko's conception. We have to assume, however, that while identifying growth as merely macroscopic change, Lysenko does not exclude some other functions, particularly in seed germination. In fact, without this reservation, incongruity can be found even in the fundamentals of his theory. It is claimed that development (vernalization) is independent of growth on the time scale, yet, on the other hand, it is persistently emphasized that "the process of vernalization does not occur in seeds in which the embryo has not begun to grow", thus stressing the significance of the conversion of a seed (resting embryo) into a plant (functionally active embryo).

Lysenko considers the germination of seeds, i.e. their preparation for vernalization, as a distinct procedure, not only as regards its cardinal environmental factors, but also as regards its physiological significance; the seed must become a plant functionally, although externally it may differ but little from a seed. The importance of seed germination for the subsequent chilling has also been emphasized by McKinney (1935): wheat plants from seeds chilled in an imbibed but ungerminated state headed 90 days after the plants from seed chilled after germination.

Long before perceptible growth of the embryo, varied internal readjustments occur in a seed. Whatever their nature, they are closely connected with a certain

physiological state of the embryo at which the capacity to grow and develop is acquired. The embryo is, however, not endowed with this physiological readiness only after germination, since ripening (milk-ripe) seeds were found (Kostjučenko, 1937 *a, b*) to be physiologically, and hence functionally, nearer to germinated seed than to fully ripe seed, i.e. the embryo which has not yet entered dormancy "must be as sensitive to vernalization as the embryo brought from that state after germination". This has been substantiated in experiments on vernalization of seed during ripening (Kostjučenko, 1935-7; Gregory, 1936*b*) while still connected with the mother sporophyte, and of unripe seed without germination (Zaruballo, 1938).

Although it has been claimed that the rate of increase in size is independent of the rate of development, and that the plant may grow, but not develop, yet from what is known regarding the process of pre-sowing or after-sowing development it can be inferred that the development, at least of the first two phases, can be maintained only at a certain minimum of growth and that non-growing seeds cannot be vernalized. It is still not clear, however, whether these facts suggest that physiologically the development processes cannot be completely separated from growth, and development is only relatively independent of growth (Razumov, 1935), or whether they indicate that the conditions required for development are at the same time more or less satisfactory for growth, i.e. the range of these conditions is wider and, as it were, includes the conditions required for development. The experimental data are too scanty to confirm Razumov's statement (1935) that development must be more rapid in green plants under conditions also favouring rapid growth than in germinated seed at a reduced rate of growth. On the other hand, it is difficult to follow Lysenko's view that growth is, as it were, one of the functions of development: "at various phases plants maintain different growth".

The relationship between growth and vernalization was investigated by Gregory (1936*c*); growth was checked by keeping two lots of soaked seed in a nitrogen atmosphere, one at 1° C. and the other at 20° C., and it was concluded that "the check to growth is not the potent factor in vernalization"; in either case, however, the seeds remained unvernallized. This might indeed be due to the lack of oxygen, equally indispensable for vernalization and for germination. The present knowledge of the true relationship between growth and development is still too meagre for definite conclusions to be drawn. }

(2) *Plant and environment*

The subsequent parts of Lysenko's theory are devoted exclusively to the individual development of plants, which is considered as a sequence of qualitative changes accumulating in the promeristem. It is universally recognized that during their lifetime plants require different environmental conditions. Thus, cereals require lower temperatures at the beginning of their development than at the end, and they pass the earliest part of their life independent of day length; later they require not only higher temperature, but also definite day length. On the other hand, cotton requires higher temperature at the beginning of its development than at the end; it is at first indifferent to day length, then requiring darkness and later

more or less long day. Moreover, not all the factors of the environment influence development, i.e. are biotic factors at a given phase. In cereals, light does not play any direct part in the completion of the first phase, i.e. light or darkness are not biotic factors in this phase, whereas light is indispensable for the second phase, and light and darkness are then biotic factors at this phase. On the other hand, development may be influenced by many factors such as light, temperature, water, pH, electricity, etc., but not all are necessarily indispensable or required by the genotype. Therefore, not all biotic factors are biological factors, indispensable for maintaining development of a phase. Lysenko (1935, 1937) strictly distinguishes "conditions of the habitat" from "influencing factors", and among the latter the factors "maintaining development". The difference between and significance of influencing factors stand out clearly in experiments on stimulation of vernalization. The period of vernalization of the first stage in wheat was appreciably shortened, when the seed were prior to vernalization germinated in pyrogallol solution (Godnev, 1935), or were vernalized in atmospheres of increased oxygen content or low concentrations (0.1–0.001 %) of ethylene, ethylene bichloride, chloroform, sulphuric ether and other gases (Eremenko, 1935). In these investigations the seed could be vernalized only under conditions described by Lysenko as indispensable; any departure from these specifications inhibited vernalization, despite the stimulative media, whereas without stimulation vernalization progressed normally.

| Plants at different phases may, and usually do, require different environmental conditions and the environment favourable for the completion of one phase may be altogether unsuitable for the initiation and progress of the subsequent phase. These facts are indeed well known, yet they have received little attention, as in environmental studies the conditions have been maintained as constant as possible throughout the experiment; if varied at all, this was done arbitrarily without reference to the plant's requirements, thus obscuring the results. |

(3) *General concepts of phasic development*

| The special requirements of plants during different periods of their lifetime suggest that they are physiologically re-adjusted with the advance in their development. "Plant development consists of a sequence of qualitatively differing étapes" or steps which Lysenko calls "developmental phases". By phases he understands only those qualitative changes in the meristematic tissue without which eventual reproduction is impossible; phases are definite and indispensable, qualitative changes upon which the inception and development of all reproductive organs and characters are based. No other qualitative changes are regarded as pertinent to development. |

Not all the developmental phases are perceptible morphologically, and not all morphological changes are a direct result of the transition from one phase to another; morphological changes may, to a certain extent, occur on the basis of some earlier phases. Therefore, by developmental phases should be understood not the formation or growth of organs and characters as such, but those qualitative changes

without which these organs and characters cannot be conceived. Therefore, the morphological manifestation (morphogenesis) of those qualitative changes which constitute a developmental phase are subsequent in time, and quite frequently considerably so, as this manifestation, in its turn, depends entirely upon the environmental conditions. From this standpoint the significance of the so-called phenological phases in recording the progress of development is practically annulled. No other methods of recording the progress of developmental phases by external characters have yet been established and attempts are now being made to detect those biochemical changes which might directly or indirectly signify the completion of a particular phase.

There is also a strict sequence of developmental phases, and without the completion of a preceding phase the subsequent phase cannot begin. If the environment is favourable for all phases but one, the plant will develop as far as that phase and no further. Thus, winter cereals sown in the spring fail to ear in the year of sowing because, owing to the high temperature subsequent to sowing, their development is arrested at the first phase; on the other hand, short-day plants cultivated under long-day conditions are arrested at the second phase. In either case, though for different reasons, development is held up until the environment is appropriately changed.

III. DEVELOPMENTAL PERIOD: THE FIRST PHASE

The first phase has now been demonstrated with a varying degree of accuracy in the development of practically all economic plants, including the short-day thermophytes, in which until recently it was only vaguely, if at all, discriminated from the second phase; indeed, some investigators were led to conclude that, as distinct from long-day plants, short-day plants require darkness for the vernalization of the first phase or that the first two phases are more or less concurrent. Actually, the technique of their vernalization (Whyte, 1933) concerns the first two consecutive phases, the first indifferent to light and the second requiring darkness; in Razumov's experiments with millet (1935), unvernallized plants grown for the first 10 days at 20–25° C. eared in continuous day after 3 days' exposure to short-day, whereas those grown at 9–10° C. required not less than 9 short-days to ear in continuous day. Evidently, the former plant had passed the first phase and then, while in a short-day period, the second also, whereas the latter failed to complete the first phase until transferred to high temperatures, hence the difference of 6 short-days.

(1) *Environmental factors*

As winter cereals fail to ear when sown late in spring, owing to the excessive temperature following sowing, many investigators conclude that temperature is a decisive or at least a dominant factor for vernalization of the first phase, and that this is a thermal induction or stimulation consisting in maintaining imbibed seed at low temperatures (cereals) or high temperatures (cotton, soybean and other thermophytes). The "decisive" role cannot yet be attributed to temperature alone.

If moisture in the seeds being vernalized or sown in the open is below a certain minimum the plant will not pass the first phase, no matter how near the other factors are to the optimum. Again, the absence of oxygen has been shown to nullify the efficiency of all other environmental factors. Therefore, each of the three factors may be equally decisive.

‡ The progress of the first, as indeed of all subsequent phases, is governed not by individual environmental factors, such as temperature, light, humidity, etc., but by mutually compensating gradations of temperature, moisture and aeration. Day length is of no importance for vernalization of the first phase, as such, but the importance of light for photosynthesis and other vital functions in growing seeds or seedlings at this stage is obvious.†

(2) Temperature

While millet, soybeans, *Perilla* and other thermophilous plants require relatively high temperatures ranging from 20–30° C. or over, other plants such as cereals, grasses, beet, etc., require relatively low temperatures. There is no clear demarcation between the two groups, for the temperature requirements vary as broadly between as within species. With wheat varieties the optimal temperature is anything between 0–3 and 8–15° C. (Dolgušin, 1935). A wide variation was shown by Avakijan (1936*b*) in tomatoes, the temperature required at the first phase by the different ecotypes varying from 8–12 to 22–25° C. The first stage is speeded up as the temperature approaches the optimum, and is retarded and eventually inhibited when it deviates in either direction. The first phase was completed in wheat (Lysenko, 1937) in 40 days at 0–2° C. and in 100–150 days at 15–20° C., while it was inhibited at still higher temperatures and below freezing point. Thus two critical limits seem to exist in the intensity of thermal factors.

There is experimental evidence that, supported by other vernalizing factors, the vernalizing temperature can be given in instalments; the total period for seed or plant being treated will thus be prolonged to compensate for the intervals but, except for interference with the physiological state of the seed or plant during the intervals, the actual time required for vernalization of the first phase may remain unchanged, a finding of particular importance in vernalization of seedlings and in recording temperatures maintained in environmental studies.

Gregory (1936*c*) found, however, that rye grains failed to vernalize under a daily alternation of low and high temperature, and earing was the later the shorter were the instalments of low temperature, thus showing that whatever changes may be initiated in seed or plants under the effect of low temperature, time is required to resume these processes when discontinued at high temperatures, i.e. the effect of temperature, as indeed of other factors, is not “trigger-like”. This would suggest a critical length of the “thermal period” (analogous with that established in photoperiodism) rather than the *devernalizing* effect of high temperature. This critical thermal period is, however, much less than 24 hr., at least for rye, as in Avakijan’s test (1935) winter rye eared when vernalized at 10 hr. high temperature + 14 hr. low temperature daily. In Gregory’s experiments high temperature was combined

with a nitrogen atmosphere, a factor debarring vernalization, and under these conditions seed vitality was impaired.

(3) *Moisture*

! Owing to technical difficulties in maintaining moisture of seed during vernalization within required limits, this factor is practically of decisive importance, as moisture tends to fall continuously. The aim of vernalization is to induce the physiologically potent embryo to complete the first phase during that period when its nutrition is least dependent upon external factors. The rate of growth and consequently of consumption of reserve nutrients increases with a rise in seed moisture; on the other hand, moisture also governs the rate of development, the first phase being inhibited at a moisture below a definite minimum.!

Moisture may be maintained within proper limits by compensative regulation of temperature, namely, with decreasing moisture temperature should increase, but such a regulation can be effected only within certain limits.

The difficulty of maintaining suitable moisture is ruled out in an after-sowing vernalization, e.g. in vernalization of planted seedlings. Owing to the difficulty of controlling temperature in the open, after-sowing vernalization is hardly practicable on a large scale. For this purpose seeds should be vernalized before sowing; the growth thereof may be somewhat retarded by moistening seed prior to vernalization in a solution of 116 g. NaCl + 14 g. KCl + 51 g. MgSO₄ per 1000 c.c. or in a buffer solution, e.g. 34.5 NaH₂PO₄ + 89.5 g. Na₂HPO₄ per 1000 c.c. (Bassarskaja, 1932, 1935), or in a solution of cane sugar (Nohara, 1936). Retardation of growth during vernalization is still open to serious objections, as the extent to which growth can be retarded without impairing the rate of vernalization is not yet known.

The discovery of the possibility of vernalization during seed-ripening (Kostjučenko, 1935-7, Gregory, 1936*b*) introduces yet another method of vernalization by maintaining appropriate conditions either during the ripening of seed while still connected with the mother plant or after harvest (Zaruballo, 1938). Using a similar method, Pobedimova (1936, 1937) reported striking success with *Cyclamen*.

(4) *Aeration (respiration)*

! Despite optimum temperature and moisture, seed cannot be vernalized in anaerobic conditions and an increase of atmospheric oxygen accelerates vernalization of the first phase (Dolgušin, 1935; Eremenko, 1935). However, the first phase in winter wheat vernalized more rapidly in almost 100 % oxygen at 0-1° C. and in 75 % oxygen at 4-6° C. than at 7-8° C. (normal atmosphere), i.e. a change in oxygen content involves a change in the optimum combination of vernalizing factors. The deleterious effect of CO₂ on seed vitality and vernalization has been shown by Eremenko (1935). !

It is obvious that, of the three factors described as biologically indispensable, moisture and aeration are also indispensable in germination. In particular, vernalization can be effected at any moisture "suitable for sustaining the seeds in an active state of germination" (Lojkin, 1936); the moisture of milk-ripe wheat seed capable

of vernalization was found to be similar to that recommended for vernalization (Kostjučenko, 1937). Thus, although the direct causal effect of temperature on the processes constituting the first phase cannot be challenged, the direct action (but not accessory through maintaining seed in a functionally active state) of the other two factors has not yet been ascertained.

The exclusion of one of the three potent factors from the environment nullifies the effect of the remainder, no matter how optimal the other two may be. A change in one of these potent factors, while the other two remain unchanged, will inevitably alter or nullify the efficacy of the environment. Therefore, the state of these three factors is not only a pre-requisite for vernalization of the first phase, but also defines within certain limits the rate of its completion and hence the period of vernalization. This period thus appears to be a variable dependent upon the state of these three factors, rather than an independent factor, as advocated by some investigators; the length of the first phase and the efficacy of vernalization will, however, be discussed later.

(5) *Other factors (light and darkness)*

The accessory or inhibitory effect of other environmental factors, as understood by Lysenko, has nevertheless raised some controversy. Referring to early environmental studies, chiefly with wheat, some investigators (Lebedinceva, 1933; Oleñikova, 1933; McKinney, 1934, 1935) suggested that, besides the factors announced as indispensable, day length has a conspicuous effect on the duration of the first phase. These claims are not evidently in conflict with Lysenko's views. On the other hand, others (Vasiljev, 1934*a*; Čailahjan, 1934) announced that the factors claimed by Lysenko as indispensable or optimal are not "those which bring about earing in the shortest possible time" and can be replaced with a varying degree of success by other factors, e.g. darkness (Vasiljev, 1934*a*) or light (Čailahjan, 1934).

Without examining these claims in detail it may be indicated that the accessory role of darkness has not been convincingly demonstrated, e.g. the comparative behaviour of the control plants growing in continuous day in Lebedinceva's test and the simultaneous earing of the control and the plants vernalized for 12 days in the increasing-day series of Oleñikova's experiment. McKinney's conclusions (1930, 1935) would appear to contradict the results obtained with the same wheat (McKinney, 1933) and to be inconsistent with his own findings that "wheat" "plants from seeds chilled in daylight headed 47 days after planting and those from seeds chilled in darkness headed 49 days later" (McKinney, 1935).

The conflicting claims of Vasiljev (Ser. 1 versus Ser. 2) and Čailahjan (the number of light hours received by the plants earing at the same time) could also be challenged in the light of their own results and, in fact, were not confirmed by Avakijan (1935), who found that, at the same temperature, the plants eared simultaneously in continuous day and after 15 and 25 short or normal days followed by a 24 hr. day. Although the plants receiving normal or 10 hr. day for the first 35 or 45 days eared the later the longer they were before being transferred to continuous

day and the shorter was the photoperiod preceding the 24 hr. day, it is evident that the cause does not lie in the accelerating or retarding effect of light on the first phase, but in the retardation or temporary inhibition of the subsequent phases in short photoperiods.

These experiments do not contradict one another or Avakijan's test, for the apparent discrepancy is not in the experimental evidence as such, but in the way the data are explained, and is understandable in the light of Lysenko's theory, even though the results are obscured through inaccurate arrangement of experiments. It is particularly important in this research to co-ordinate environmental factors as near as possible to the actual requirements of the plants, and to keep more accurate records of temperature than the daily mean.

The influence of short and long photoperiods on the time of jointing in winter cereals would appear to have been first pointed out by Wanser (1922), who stated that "there probably are for most monocotyledonous and some dicotyledonous plants at least two critical photoperiods, one for starting culm or stalk development, and another for starting heading or budding and blooming stage". Thereafter the effect of photoperiods at early growth phases on wheat and other cereals has been repeatedly studied by many investigators, but with conflicting results. The discrepancy of opinion is to a great extent due to the fact that photoperiods were studied without strict co-ordination with plant requirements and other environmental factors.

A more detailed and precise study was recently carried out at the Ukrainian Institute of Agriculture by Mackov and his associates (1936), and particularly by Eremenko (1936, 1938), who showed that, in spite of the fact that the temperature was favourable for the second phase, wheat plants did not respond to prolongation of day length during the first 8 to 10, or even 15 to 18 days in strong winter wheat (after vernalization) after the first developmental phase; Eremenko (1938) concluded that "it should be assumed that in wheat there exists a biologically important transitional stage of development between yarovization [the first phase] and the light stage [the second phase]". It should be noted, however, that the data quoted by these investigators do not show an unmistakably inductive effect of darkness (short day), although the retardive effect of light (long day) seems to be sufficiently obvious, showing thus that the failure to respond to photoperiods should not be sought in the physiological impotency of young plantules, as affirmed, for instance, by Callahjan (1937). If in further study this supposed "transitional" phase is substantiated, the existence of this phase will clear up the discrepancy of opinion, as well as emphasize the precision required in the arrangement of an environmental study, and the importance in this research of co-ordinating environmental factors as closely as possible with the actual requirements of the plants.

(6) *Length of the first phase*

In comprehensive studies of world collections of the chief agricultural plants, e.g. *Triticum* (Dolgušin, 1935), *Hordeum* varieties (Razumov, 1934, 1935), *Hordeum pallidum*, *Avena*, flax, etc., the response of forms to vernalization and hence the

length of the first phase could be related to their eco-geographic rather than to their botanical origin.

The response to vernalization is undoubtedly connected with the type of the first phase and thus is a biological character acquired in the evolutionary differentiation of forms. Yet it is not less connected with the after-sowing conditions and the place of test. In other words, the response to vernalization or its after-effect (shortening of the vegetative period) and the length of the phase are not identical, for the after-sowing behaviour of plants is governed by the environment which has a different effect on the development of vernalized and unvernallized plants when sown simultaneously, as may be inferred from the following experiment. Plants of Bokhara barley grown from vernalized and unvernallized seeds at low temperature and from unvernallized seeds at high temperature eared simultaneously after 47 days. It might be concluded that this barley did not respond to vernalization and hence that the first phase is very short or even "absent", had not the vernalized plants grown at high temperature eared after 35 days. Evidently, this was due to the high sensitiveness of this barley to temperature, both in the first and subsequent phases; high temperature retarded the first phase, but speeded up the second, while low temperature speeded up the first, but retarded the second (Razumov, 1934).

Some other environmental factors may also have a marked effect on the response to vernalization, plants proving to be more sensitive the more is known of them. Vernalized and unvernallized plants of spring wheat headed 5-9 days earlier in Hibiny (24 hr. day) than in Pushkin (18 hr. day) (Kostjučenko, 1934). The heading of vernalized rice plants was late and only slightly ahead of their controls when grown in the natural day of the Leningrad summer and conspicuously earlier when grown for the first 30 days in a 12 hr. day (Avakijan, 1936a). Therefore, low temperature combined with a shorter day in one case and with a longer day in the second reduced or even nullified the advantage gained in vernalization, as in either case the after-sowing conditions retarded the second phase (the first after sowing) in vernalized plants and speeded up the first phase in the control. Therefore, the evaluation of the length of a phase by the relative efficacy of vernalization (the difference in time of flowering or earing) when the vernalized and unvernallized plants are sown together is subject to serious objections, as any results thus obtained are invariably connected with after-sowing conditions and hence with time of sowing and place of test (Dolgušin, 1935, and others).

A more accurate idea of the length of the first phase can be gained when seeds vernalized for a varying number of days are grown after simultaneous sowing under conditions suitable for subsequent development. Some investigators still fail to realize that vernalization is not a stimulation or induction speeding up the entire developmental period, but only a pre-sowing completion of part of the developmental period, and, as such, has no direct effect on the vegetative period, which is controlled by the environment under which the plants are grown. Naturally, the pre-sowing completion of part of the development places the plant in a new relationship with the environment, and thus the effect of vernalization is analogous to that of a change in time of sowing.

The correct understanding of the effect of vernalization would dismiss altogether the fruitless attempts to ascertain whether vernalization shortens the time between sowing and maturity, or whether "when the period of low temperature treatment is included the time taken to flower remains constant" as compared with the vegetative period of the unvernallized control.

Reference to the after-sowing conditions is just as important in the evaluation of vernalization as an agronomical method. To gain the fullest possible advantage from vernalization of the first phase new optimal dates for sowing vernalized seeds must be established.

Yet another aspect deserves particular attention. Kostjučenko (1935) found that vernalized and unvernallized wheat plants from the seed of Hibiny ($67^{\circ} 44'$ N. lat.) reproduction eared at the same time, whereas only the vernalized plants from the seed of a Ganja ($40^{\circ} 41'$ N. lat.) reproduction produced heads. This striking behaviour could be traced to the environment in which the seeds were reproduced. It was assumed that the seeds during ripening were able to complete part of their development when reproduced in Hibiny (cool summer nights), but that they failed to do so in Ganja (warm summer). As these conclusions have been fully confirmed (Kostjučenko, 1935-7; Gregory, 1936c), this fact acquires particular importance in the study of the individual development of plants and the related theoretical and practical problems and calls for a thorough revision of the conclusions hitherto made regarding the relative response of plants to vernalization and the degree of their lateness.

This discovery also suggests that if, say, in winter cereals the first phase can under certain and perhaps special circumstances be completed during seed ripening, in other plants a full or partial completion of this phase may occur each year during seed ripening. There seems to be little or no doubt that the phase which for the sake of convenience is described as the first phase is not the first phase of the entire development of a plant, but is merely the first phase of many, preceding and succeeding phases, at which development is usually interrupted in winter cereals, to be continued after seed germination if the environmental conditions permit. There seems to be little doubt, as pointed out by Gregory (1935), that "considerable development has of course already occurred in the embryo during fruit formation", and the potential length of the vegetative period and the general behaviour of plants after sowing "depend on how far this embryonic development has proceeded".... "One may, however, postulate a stage in the development of these plants comparable with puberty in animals". This view has received much support from the results obtained recently by Abakumova (1938), which she vaguely attributed to "photoperiodic and temperature after-effects". In these experiments with spring and winter wheat, when the daily photoperiods were shortened, earing was delayed longer in winter forms (11 to 25 days) than in spring forms (1 to 7 days). However, in the subsequent year under long photoperiods the plants from seeds which ripened in shortened photoperiods eared earlier than those from seeds ripened in longer photoperiods, the delay in the latter group being greater with winter forms (13 to 24 days) than with spring forms (0 to 2 days).

IV. DEVELOPMENTAL PERIOD: THE SECOND PHASE

(1) *Discovery of the second phase*

Even as early as 1928 Lysenko postulated that the life period of cereals from germination to jointing comprises two phases, the first requiring low temperature and the second high temperature with certain other factors. Lebedinceva (1933), McKinney (1935) and others also detected a second phase in cereals with specific requirements as to temperature and day length. The most convincing evidence, however, was provided by Meljnik (Lysenko, 1932*e*) in experiments with vernalized and unvernallized winter wheat grown at high temperatures in a 10 hr. or continuous day; only the vernalized plants grown in continuous day headed.

In another experiment, vernalized plants grown first in continuous day for not less than 20 days eared as rapidly in a 10 hr. day as those kept in a continuous day throughout, i.e. after 20 continuous days long or continuous light is not obligatory, and the long day is indispensable only for a definite part of the period following the first phase. This part of the developmental period was termed the *photo-phase*, but in order to prevent a misleading conception of a prevailing effect of light it is referred to here as the second phase.

It might be inferred from these experiments that the internal changes constituting the first phase alone are insufficient to make the plants reproductive; however, until these changes are completed the plants are unable to begin the subsequent phase, a strict sequence of phases being maintained.

This phase has also been demonstrated in soybean, millet and other thermophilous short-day plants, which at this stage have their own requirements as to light and temperature.

(2) *Photoperiodism, photoperiodic induction (after-effect), second phase*

Although the causal effect of light was first discerned by John Ray in 1685 and demonstrated by Tournois in 1911-12 with time of flowering in *Humulus japonicus*, it remained for Garner & Allard (1920) to demonstrate the profound effect of day length on flowering and to announce the principles of a classification based upon the formative effect of light, which is still held with some modification.

Garner (1933), summarizing investigations on photoperiodic responses, concluded that "in many species representing both the long-day and short-day types, a variation in day length of not more than one hour (or even less) constitutes the critical range, on the two sides of which definite contrast in response is obtained", namely, "on the one side the plant flowers readily, while on the other it tends to remain in the vegetative stage". Accordingly, "in the short-day group flowering is initiated by day lengths shorter than the critical and in the long-day group by day lengths in excess of the critical, and the essential characteristic of the less sensitive or intermediate group of plants is that they possess no clearly defined critical light period".

Eghiz (1928) found, however, that short-day plants grown in a short day for the first 10–20 days and then in long day flowered quickly, whereas plants grown invariably in long day did not flower. This discovery, anticipated by Garner & Allard in 1920, became known after Maximov as the “photoperiodic after-effect”; virtually the same phenomenon was studied by Ljubimenko since 1927 and others as “photoperiodic induction”. As the treated plants showed no morphological changes it was postulated that a short day in the short-day plants and a long day in the long-day plants induce some chemical changes, which later bring about the differentiation of the meristem.

Lysenko gave a quite unexpected approach to the photoperiodic classification of plants. Virtually, “in long-day plants flowering is initiated by day lengths in excess of the ‘critical’ photoperiods” (Garner, 1933), and the earlier the longer is the day. Thus, “no requirements as to photoperiods are inherent to long-day plants” (Lysenko, 1932*c*); these plants require a continuous day to complete one of their developmental phases and can but tolerate a daily alternation of light and darkness, when the dark period is not in excess. Moreover, in some early experiments with maize, cotton and some other short-day plants (Lysenko, 1931*a, b*), plants grown from seeds vernalized in darkness flowered earlier than those from seeds vernalized in short day and the control. As, in addition, summer photoperiods speeded up development in vernalized plants and retarded it in unvernallized plants, it is claimed that short-day plants require not a short day, but darkness to complete one of their developmental phases and only tolerate daily alternation of darkness and light, when the latter is not in excess of a critical duration. The fact that continuous darkness, but not a short day, is required could be shown only through vernalization, as the absence of light in environmental studies for a longer time would prevent photosynthesis and thus impair the vitality of the plants.

Following upon this conception, Dolgušin (1932*a*) investigated the “nature” of the photoperiodic after-effect in millet. Only the plants first given 12 or more short days flowered in continuous days and did so practically at the same time; those transferred from continuous day to short day flowered in 22–46 days after planting, according to the time of transference to short day. Nevertheless, neither did the delay exceed the exposure to continuous day, nor did the time required to flower increase with longer exposure to continuous day. It is held, therefore, that no inhibitory substance had been formed in continuous day, and that the photoperiodic effect consists in that darkness is required for millet to complete one developmental phase, defying thus the antagonistic after-effect of the long day on short-day plants, and the short day on long-day plants. Later, Čailahjan (1935), Razumov (1935) and others reached similar conclusions.

(3) *Environmental factors*

In a test of photoperiodic after-effect (Dolgušin, 1932*b*), darkness at a lower temperature had no effect on the time of reproduction, and the efficacy of darkness increased with a rise in temperature. In other words, darkness became effective only when combined with some other factors, in this case with adequately high

temperature. Thus, if we have to speak of an after-effect we must consider not photoperiodic induction alone, but "thermo-photo-periodic" induction, or more correctly induction by all the component factors required for the second phase.

Not only the short-day plants show definite requirements to day length and temperature for the completion of their second phase. In Razumov's (1935) experiment with cereal plants from vernalized seeds, plants grown in long day throughout and those grown first in a long day for 18 days at high temperature headed at practically the same time, and earlier than those receiving 18 long days at a low temperature, the delay within the same species appearing to be greater in the more winter varieties. Finally, the plants grown under short-day conditions throughout were also delayed; this delay again varies with the variety, being apparently greater in northern strains than in southern. Therefore, not only different plants but different strains of the same plant show their own characteristic requirements as to day length and temperature.

(4) *Light and darkness*

We cannot agree literally with Lysenko (1932*c*) that "by darkness must be understood not only the absence of light as perceptible to our eye, but also as an independent factor". In Razumov's study (1935) of the photoperiodic effect of different regions of the solar spectrum, a partial or complete substitution of darkness by monochromatic light of different quality, but of equal intensity, showed that the long-wave light, mainly red rays, acted as light both in long- and short-day plants, whereas short-wave light (green, blue and violet, alone or combined) was equivalent to darkness. Katunskii (1937) and Murneek (1937) made a similar conclusion for short- and long-day plants; all sections of the spectrum produced a photoperiodic effect, but to a degree which varied and seemed to be connected with their absorption by the leaves.

Co-ordinating his results with Lysenko's theory, Razumov (1933) concluded that it is not merely the absence or presence of light which governs the completion of the second phase, but the absence or presence of the long-wave region of the solar spectrum.

In this connexion Razumov's investigation (1935) of the photoperiodic effect of light intensity on short-day (millet) and long-day (oats) plants deserves particular notice. Millet from Pamir, Bokhara and Irkutsk grown in a short day with additional light of from 0.5 to 10 luxes headed at the same time as the short-day control and 40-44 days later in the 24 hr. day; types from Voronezh and Bezenchuk headed at the same time as the short-day control only under short day + 0.5 lux illumination, the remaining series being retarded; millets from Persia, Korea, Mongolia and Central China were retarded in all variants with additional illumination, as compared with the short-day control.

Oats also showed a different reaction to light intensity; Palestine types headed at the same time as the continuous day control, in short day with 60 lux illumination and only 3 days later in short day with 10 lux illumination, whereas Finnish types did not head with 10 lux illumination and headed 21 days later with 60 lux illumination.

Therefore, plants show requirements "not to light or darkness, but to a definite light intensity, varying with variety and species". "The presence within the same species of a gradual transition in light requirements, i.e. in degree of long- and short-day habit, makes it possible to show that, if on the one hand there are plants with a clear-cut need for very weak light (Mongolian millet) or very strong light (Finnish oats), then on the other hand these two consecutive series of long- and short-day plants converge in their light requirements for the second phase"; Bokhara millet, completing its second phase under 10 lux illumination, responded to it as to darkness, and Palestine oats, completing its second phase in the same illumination, responded to it as to light. "Therefore, the principal difference between long- and short-day plants disappears," and these "may be arranged in a single descending series in which it would be impossible to delimit the different groups in respect of photoperiodism".

Undoubtedly "light" and "darkness" must be relative conceptions, but Razumov seems to overlook the difference between the light requirements of long-day plants at the second phase from the light tolerance of short-day plants at the same phase. Although the critical values may overlap, yet these are limits of two diametrically opposed groups, the one requiring a light intensity not below a critical intensity and the other the absence of light, which is only tolerated if the intensity does not exceed a critical value.

(5) *Other factors*

Although the second phase has been studied in many plants the experimental data available permit us to form only a rather broad conception as to the environmental factors affecting this phase; undoubtedly, other factors besides day length and temperature, for example, moisture (particularly in the growing seeds), nutrition (particularly for grown plants), aeration, etc., might be, and are of significance, but there is no evidence to show which of these are "biologically indispensable" to plants at this phase.

At a glance it would appear that the extensive data accumulated in the study of photoperiodism might be used for that purpose, but it has been pointed out that the photoperiodic response as defined in photoperiodism and in the theory of phasic development are not identical conceptions. While refraining from any discussion of the adequacy of the photoperiodism outlook, it should be pointed out that the fact that with development a plant may change in its relation to light has been overlooked in studies of photoperiodism. In fact, by maintaining an invariable photoperiod throughout most of a plant's life a kind of compensating photoperiod suitable for all phases could, perforce, be established, as by Čeljadinova (1937), who found that deviation in day length in either direction from a 10 hr. day delayed flowering in *Arachis hypogaea*. How erroneous conclusions based upon experiments so arranged may be can be inferred from the conflicting opinions as to the photoperiodic class of tomatoes. Yet even with an approximate co-ordination of day length with actual requirements, as maintained by Mihašlova (1936), an increase in day length from 9 to 12 hr. or more before budding retarded time of flowering,

while shortening of the day after bud formation had a similar effect. Thus, in Garner's terminology, the tomato is neither a short-day nor a long-day plant but may be described as a *short-day → long-day plant*. Furthermore, the existence of a rather large group of so-called "neutral" plants is hardly congruous with the sensitive constitution of plants and seems to emphasize the imperfection of experimental evidence hitherto obtained for this classification.

Although in investigations of the photoperiodic after-effect (induction) this aspect has been more satisfactorily approached, yet in both the causal effect of day length was studied independently of other factors. The importance of this error has been clearly shown by Gilbert in 1928 with *Xanthium pennsylvanicum*, which was, so to speak, converted from a long-day type (at low temperature) to a short-day type (at high temperature).

(6) Length of the second phase

| The photoperiodic adaptation of plants (in Ljubimenko's nomenclature) had shown that not all long-day plants are equally "long-day", nor all short-day plants equally "short-day"; their photoperiodic response varies with biotypes (Oakley & Westover, 1921) even within the same species, and this variation was found to be closely connected with phylogenesis of the species (Sinskaja, 1937).¹ To what extent does this photoperiodic adaptation of plants hold true in the study of photoperiodic response at the second stage?

The correlation between the length of the second phase and geographical origin was detected by Koreiša (1935) in lucernes grown in natural and 10 hr. days respectively, yet no correlation was detected by Dolgušin (1935), as Finnish and Abyssinian wheat eared 10 days earlier in continuous day than in the seasonal day of Odessa.

In a study of the sensitivity of different barley varieties to day length (Razumov, 1935), those of southern and northern origin eared most rapidly in a 24 hr. day, but earing was more delayed in the northern varieties than in the southern when the length of day was reduced. Therefore, in the cool northern summers (67° 44' N. lat.) northern varieties showed a greater response to longer day than the southern, but does this mean that the southern varieties are less sensitive to light? Undoubtedly, the environmental requirements of southern biotypes must differ from those of northern biotypes and not only in respect of day length. The experimental evidence is still too scanty for any definite conclusions to be drawn on this question, but two important aspects must be emphasized.

The progress of the second phase is governed by a definite combination of environmental factors varying for different biotypes; investigations of the effect of duration, quality and intensity of light, without specifying the other factors, may be very misleading, as may be inferred from an experiment on northern barley (Razumov, 1934). Vernalized plants grown in a 15 hr. day at a high temperature and in a 24 hr. day at a low temperature eared in 35 days; this does not mean, however, that this barley is insensitive to day length, for when grown under a 24 hr. day at a high temperature earing occurred in 29 days. The temperature factor, therefore,

is of importance for the second and subsequent phases; the requirements undoubtedly differ with biotypes and hence deficiency in temperature will modify the effect of the light differently. In fact, when vernalized plants of a northern and a southern barley were grown in a 24 hr. day (Razumov, 1934), heading at the high temperature was practically simultaneous, but the northern type was 6 days earlier at low temperature, i.e. the fall in temperature had a greater effect on the southern type.

It is particularly important to know exactly *when the second phase begins* and *when it ends*, as some plants change abruptly in their requirements and hence in their relationship to the environment. The method of diagnosis suggested by Bassarskaja (1934*a, b*, 1936) for the second phase is but a first step in this direction.

The method of vernalization of the first two phases offers great opportunities to investigators, but so far its application presents considerable difficulties, particularly for plants requiring long day and high temperature at the second phase, i.e. conditions which stimulate rapid growth of seed. On the other hand, the application of a chemical method of retarding growth during vernalization suggested by Bassarskaja (1932, 1935) and others is open to serious objections, as already stated.

V. DEVELOPMENTAL PERIOD: THE SUBSEQUENT PHASES

In reports published since 1928 Lysenko speaks only of the first two phases, which cover the lifetime of annual plants to the inception of the floral primordia or thereabouts. Although some investigators have laid special emphasis on the significance of the first two phases, yet of no less importance in environmental studies must be the relation of plants at subsequent phases to the environment, particularly in polycarpic plants.

Razumov (1935) reported that complete sterility could be induced by changing the day length, and that this was associated with enlargements of the sterile tip of the stem. Of new shoots usually arising from the axils of the leaves nearest to the sterile ear, or from the sterile inflorescence, as in *Poa* and *Agrostis*, only those formed from promeristem produced floral organs. Whatever may be the cause of the origin of new shoots, it is evidently connected with cessation of development in the tip, for sterility itself suggests that the developmental cycle was left incomplete. The period from seed setting to full maturity was also found by many investigators to be closely connected with temperature.

Undoubtedly, after completing the second phase, plants undergo further internal changes, each requiring different, but definite environmental conditions. Lysenko (1934) speaks of five developmental phases in cereals, but gives no idea as to the nature of the other three.

(1) *The third phase*

This was first revealed by Kiričenko (1934*a*) and Kraevoï (1935). Vernalized plants of winter wheat were grown for the first 44 days either in continuous day (ser. A), 12 hr. day (ser. B), or 10 hr. day (ser. C); 12 plants from each series were

later placed for the following 34 days in photoperiods varying from 2 to 12 hr. Series B and C did not head; primordia were more or less differentiated in all plants of ser. B, except those in 2 hr. day, whereas not a single plant of ser. C had produced floral rudiments. Plants of ser. A eared almost simultaneously, but those grown for the last 34 days in 2 and 4 hr. photoperiods set no seeds, as the pollen was sterile (Kraevoi), suggesting thus an interference with gametogenesis.

The third phase in wheat was further investigated by Kiričenko (1934*b*) with similar results. All plants eared, but no fertile pollen or seeds were produced by plants transferred on completion of the second phase to a 4 hr. day. When these sterile plants were given 2-4 continuous days they produced fertile pollen and seed, i.e. they required a relatively small amount of light for producing normal gametes.

The fact that plants on completing the second phase can develop further in 8 hr. day, which is inadequate for the second phase, suggests that they enter into a new state with somewhat different photoperiodical requirements, and this was identified as the third phase.

It remains obscure, however, why plants in 6 hr. day produced normal pollen and seed as rapidly as in a longer day. This seems to diverge from Lysenko's conception as to the meaning of long or short days in the life of plants. If his conception holds true, the third phase seems to consist of two subphases, one requiring darkness and tolerating some light, and a second requiring light and tolerating darkness. The photoperiods maintained in these experiments could indeed accelerate one subphase at the expense of the other.

At a glance this postulation seems to be inconsistent with the universally accepted fact that cereals come rapidly into ear when grown invariably in a day supplemented with electric light; it might nevertheless find support in more detailed environmental studies, particularly in view of a lower sensitiveness of southern races of cereals to prolongation of day length, as in these the supposed requirement to darkness must be more marked. (See Addendum.)

VI. PHYSIOLOGICAL "CAUSE" OF VERNALIZATION

In the previous parts differentiation of promeristem was quoted as evidence of the completion of the first or first two phases. Cereal plants (Avakijan, 1935) grown unvernallized at high temperature, or first at low temperature (vernallized) and then at high temperature remained sterile in short day; but when transferred to long day, the unvernallized plants remained sterile, while the vernallized formed floral primordia and became reproductive, thus showing that, despite no immediate morphological difference, the promeristem had differed functionally in these plants.

It is true that the inception of floral organs, progressing parallel with the second phase, could not yet be separated as, at least in long-day plants, the high temperature and light required at the second phase promote at the same time differentiation and growth of the primordia. The only evidence that the concurrence of these two processes is incidental is provided by the possible vernallization of this phase, at least in some plants, before any differentiation of the promeristem (Whyte, 1933).

(1) *General conceptions*

In anatomical studies (Meljnikov, 1936) the cells situated above the growing point were found to form chlorophyll in minute plastids parallel with the differentiation of the growing point and in close relation with day length and temperature. As in unvernallized plants this chlorophyll was not found, at least in sufficient amount, it is held that, under the conditions favouring the completion of the first phase, certain photosynthetic chromidia and plastids are formed in which the chlorophyll is elaborated under conditions favouring the second phase. Filippenko (1936) also noted increased chlorophyll content in leaves of vernalized wheat, but this excess was retained throughout the test in these plants. All that can be said with any degree of certainty is that some internal readjustments preceding morphological differentiation in the promeristem constitute the essentials of developmental phases, and, as Bassarskaja (1934 *a, b*, 1936) showed, concern the physical and chemical state of the protoplasm. Actually, the promeristem after vernalization of the first phase plasmolysed at a higher salt concentration and showed a marked difference in staining reaction, while plasmic permeability was increased (Filippenko, 1936).

In experiments with wheat, of plants vernalized for a varying number of days and grown at high temperatures (Lysenko, 1932 *e*), those vernalized for 21 days and over eared at the same time, while those vernalized for shorter periods failed to ear and remained similar in appearance to the controls until subjected to low temperature for the number of days they lacked previously, the time required for additional vernalization being independent of the length of the interval between the end of the first and the beginning of the second treatment. Virtually, the same phenomenon was observed in Meljnik's environmental studies (Lysenko, 1932 *e*) and Biddulph's histological study (1935) on the photoperiodic effect. This suggests that those changes which eventually, on their completion, alter the "quality" of the promeristem proceed in a strict rotation, i.e. the changes constituting the second phase cannot begin until those identified with the first phase are completed; moreover those internal readjustments which constitute a phase are gradual, and "quantitative", so to speak, cumulative and retainable, and yet their completion makes the cells functionally and qualitatively different. This has also been detected biochemically (Bassarskaja, 1934 *a, b*, 1936); the ability of the meristematic tissues to stain blue increased with vernalization and reached a climax on completion of the first phase. Again, the growing point of plants grown after vernalization in 8 hr. days for 8 months stained blue until the plants were transferred to conditions favouring the second phase (Bassarskaja, 1934 *a, b*). Therefore, they did not lose the quality acquired during vernalization, although the environment prevented them from developing further.

All the environmental and biochemical studies suggest also that the "quality" acquired by the promeristem is also retained and hence irreversible, i.e. a cell possessing the "quality" of one phase cannot be returned to the initial or preceding phase.

"Reversibility" of development has, however, been frequently reported (Klebs, 1918, Ljubimenko, 1933, 1934; and others). By an abrupt change in photoperiods

plants were forced to cease flowering (reproductive state) and to resume growth (vegetative state), which was followed by a secondary flowering.

Strictly speaking, reversibility assumes that a tissue has the faculty to proceed in two diametrically opposite directions as affected by environmental factors. In other words, since the promeristem is the centre of the phasic changes, one has to assume that the promeristematic cells are endowed with the faculty to undergo both the progressive changes maturing the tissue and the regressive changes rejuvenating the same tissue.

In the above examples there is no evidence of rejuvenescence of the tissue; in fact, in all cases "reversibility" could be described as enlargement of the tip or flowering of axillary stems, i.e. one of the less advanced axillary buds having resumed development as the environment was changed. Therefore, there might be a kind of "rejuvenescence" of a plant as a whole, but this has nothing in common with true reversibility.

"Reversibility" of the first phase due to over-vernalization might appear to be more probable. Vernalization for 20 days retarded budding in cotton by 2-5 days, as compared with a shorter vernalization (Gavrilova, 1935) and the iso-electric point then returned to its original value. While it remains to be proved that the reverse shift of the i.e.p. of albumino-lipoids implies reversibility of the first phase, the fact that the germination of seeds vernalized for 20 days was abruptly reduced suggests that the real cause of delayed budding is connected with partial or complete destruction of the growing embryo during prolonged vernalization, as suggested by Timofeeva (1934b).

Čepikova (1934) reported reversibility (delayed flowering) induced by prolonged vernalization in red clover, which was, however, repeated in the subsequent year (1935). It is not unlikely that the retardation was due to the impaired vitality of the germinated seeds, while the recurrence of reversibility in the second year seems to suggest a relationship between the end of one developmental cycle and the beginning of the other in the case of perennials. Possibly Ljubimenko's example (1933) is more suggestive; vernalized wheat plants treated with X-rays failed to ear, those untreated or given smaller doses eared. As the latter plants lacked vigour, failure to ear may be due merely to a lethal effect of X-rays on the cells previously subjected to vernalization. On testing vernalized seeds after 12 months' storage, Lebedev (1936) showed that "rejuvenation", i.e. the loss of the properties acquired in vernalization, was invariably associated with destruction of the growing point.

Therefore, it can be concluded that reversibility of the first phase has not been proved experimentally, and there can be little doubt as to the real nature of the "reversing" effect of over-vernalization and seed drying.

(2) *Hormones*

Lysenko himself has not investigated the physiological "causes" of reproduction, but he indicates their complexity, as well as the fact that "phasic changes occurring in a plant or its organs are irreversible"; he has, however, refuted the hormonal theory

suggested by Maximov and experimentally shown by Krasnosel'skaja-Maksimova (1931), with the substitution of a part of the endosperm of imbibed grains by pulp prepared from the endosperm of vernalized and unvernallized grains of spring and winter wheat. Pulp from spring wheat failed to accelerate earing of spring and winter wheat and oats. Winter wheat pulp had a retardive effect on earing and inception of floral primordia; pulp from grains chilled for 6 weeks had, however, a similar effect. It was postulated that "winter wheat contains a substance of a hormonal nature which retards reproduction", and that heading of winter cereals after vernalization is associated with the destruction of this "winter hormone" by low temperature.

Serejskij (1934) failed to substantiate these hypotheses, the pulp introduced having no effect on time of earing. In another experiment (Serejskij, 1934) in which the halves of vernalized and unvernallized grains were exchanged in various ways, the time of heading also remained unchanged. Moreover, plants from "compound grains" with a single embryo were taller and heavier than those from "compound grains" containing two embryos, showing that the endosperm, while apparently affecting growth vigour, had no effect on rate of development; hence the physiological "cause" of vernalization should be sought not in the endosperm, but in those changes which occur in the embryo, a fact confirmed by vernalization of an excised embryo grown on nutrient agar (Gregory, 1936*a*; Konovalov, 1937*b*).

The discovery of a hormone (blastanin) secreted by imbibed endosperm, and its behaviour during germination and vernalization led Cholodny (1936*a*) to postulate that the physiological cause of vernalization consists in an accelerated absorption of blastanin by the embryo; as growth is practically held up, owing to low temperature and deficient moisture, this hormone is not expended and its accumulation in embryonic plants results later in accelerated development of vernalized plants.

Following upon this hypothesis, Cholodny (1936*b*) replaced the effect of low temperature by a direct increase of the phytohormone content in the embryo. The growth vigour of treated oat plants was affected, but only oat plants treated with pulp containing blastanin eared 11-12 days before the control.

Tovarnickij (1937) attempted to "hormonize" wheat and vetch by soaking their seeds in urine solution and yeast extract. In all cases, and particularly when hormonization was followed by vernalization, the growth rate was accelerated in wheat, but no change in time of earing due to hormonization was recorded.

Neither Serejskij's experiments (1934) nor those on vernalization of the excised embryos (Gregory, 1936*a*; Konovalov, 1937*b*) could strictly speaking be opposed to this hypothesis, as in these experiments care was not taken to prevent a possible transfer of blastanin from the imbibed endosperm into the embryo. Nevertheless, this hypothesis conflicts with the bases of phasic development and with all that is known of vernalization of many plants at high and low temperatures and is not substantiated in recent investigations (Serejskij, 1937; du Buy, 1937).

(3) *Enzymes*

Tolmačev (1929) attempted to interpret the physiological effect of chilling on imbibed seeds of winter wheat and beet in terms of a certain "vital phase in their stem-plasm" associated with the products of metabolism in the imbibed seeds when chilled. This functional readiness of the "stem plasm" was found to be closely associated with marked increase in amylase and catalase content.

Demkovskii (1932) claimed that in winter wheat seeds under vernalization the proteolytic activity rose continuously, whereas the amylolytic and catalytic activity rose for the first 25 and 30 days, then fell and rose again on the 35th day. Richter (1933) reported that in seeds and seedlings of winter wheat under vernalization the hydrolytic enzymes increased continuously, but peroxidase and catalase attained a climax followed by an abrupt fall.

Enzymes were studied in lupin seeds grown at 6–7° C. for 12 days or at 4–5° C. for 15 days by Sapožnikova (1935). Only in the former series the plants were vernalized and flowered earlier. The content of "halactanase" (enzyme acting on carbohydrates) in the high temperature series rose continuously and was higher than at low temperatures. The protease content also rose continuously and reached a maximum on the day of sowing at high temperatures, but varied indefinitely at low temperatures. The catalase and peroxidase content under high temperatures rose continuously and was much higher than at low temperatures.

Therefore, of the enzymes studied, there appears to be a suggestive difference between vernalizing and non-vernalizing seeds only in the activity of catalases and peroxidases, but how far these enzymes directly concern the progress of the first phase has not been decided. Unfortunately, enzyme analyses were not always parallel with the test of the efficacy of vernalization. It must be noted that the content of these enzymes was conspicuously higher in seeds capable of vernalization (milk-ripe and germinated) than in dry seeds incapable of vernalization (Kostjučenko, 1937*a, b*).

The catalytic effect and changes in sugar content (Volkov, 1928, and Timofeeva, 1930, quoted by Krasnosel'skaja-Maksimova, 1931), soluble and insoluble carbohydrates (Savostin, 1934; Sapožnikova, 1935), respiration (Richter, 1933; Sapožnikova, 1935) during vernalization were much less suggestive.

(4) *Protoplasm*

Many internal readjustments must indeed occur in the promeristem during vernalization, but not all necessarily concern the advance in development; indeed, some must reflect growth vigour and other functions which have no direct bearing on development. A definite set of changes must, however, be completed in embryonic tissues during vernalization to make the embryonic plants "qualitatively" different; Bassarskaja (1934*a, b*, 1936) has shown this with the reaction of vernalized and unvernallized promeristem to various stains. Although supplying little information regarding the exact nature of these changes, her results suggest that it is the physical and chemical properties of the protoplasm which are concerned and, as a matter of course, form the basis for diagnosis.

The method of discrimination between vernalized (first phase) and unvernallized tissues may be briefly described as follows: Longitudinal sections of the promeristem are treated first with 5 % FeCl_3 and then after drying with 5 % $\text{K}_4[\text{Fe}(\text{CN})_6]$; or the epidermis of upper leaves are treated with 0.1 % mixture of methyl and methylene blue. With this treatment, vernalized tissues stain blue whereas unvernallized tissues either do not stain at all, or stain differently. Richter (1933) had similar results with fuchsin and methylene blue. This method has been used with wheat, barley, millet and other plants, but it apparently cannot be universally applied without modification to all plants, as the $p\text{H}$ varies broadly with species, e.g. the vernalized meristem of vetch stained yellow and the unvernallized red, when treated with chlor-phenol-red solution, the $p\text{H}$ varying from 5.2 to 6.8 (Bassarskaja, 1936).

Richter (1933), using toluidine blue as a dye, traced the difference in staining reaction to changes in albumino-lipoids. When treated with solutions of phosphatic buffers ($p\text{H}$ ranging from 3.8 to 9.2), sections of unvernallized embryos did not stain in the most acid solutions, whereas those of vernalized embryos stained readily. The ability to stain increased with increasing $p\text{H}$ until above 8.3 unvernallized embryos stained more intensely than vernalized; staining is thus greater with vernalized embryos at a lower $p\text{H}$ and with unvernallized at a higher $p\text{H}$. Richter (1934) treated wheat and cotton embryos with buffers, then stained in 1 % eosin and methylene blue and returned to the buffers; vernalized embryos (i.e.p.=below 3.8) stained blue, unvernallized (i.e.p.=above 4.9) bright red in acid buffers; i.e. the changes in seeds under vernalization concern the i.e.p. of albumino-lipoids.

The physical and chemical changes occurring in protein lipoids as a result of vernalization were studied by Filippenko (1936), who found that the soluble protein in vernalized plants coagulated at lower temperatures than in unvernallized plants. At the same time the plasmic permeability increased.

The same author also found a relative increase in amide N content in the leaves of vernalized wheat plants, which, however, was maintained through the vegetative period. The detailed nitrogen content, namely, total N, insoluble and soluble protein N, amide N and ammonia N was studied by Konovalov (1937 *a, b*) in seeds under vernalization and in plants vernalized at the tillering stage, as compared with seeds germinating at 25–26° C. and with plants grown at high temperatures throughout. At high temperatures a considerable part of the proteins was decomposed, but not in seeds or plants under vernalization; on the contrary an increased content of non-protein N in imbibed seeds was later much reduced during vernalization, and accompanied by a parallel increase in soluble protein N. Apparently, during vernalization nitrogenous substances are re-formed, this being the principal difference between the changes in nitrogenous substances in seeds under germination and vernalization.

The exact nature of the changes identified with the completion of the second phase has not yet been studied, while the evidence obtained in the study of the effect of photoperiods on the internal environment of plants in photoperiodism cannot be used with certainty in connexion with the second phase. Differences in

"quality" between the tissues which had completed the first and first two phases respectively were demonstrated by the staining reaction of the lower epidermis of leaves (Bassarskaja, 1934a, b, 1936). Solutions of 0.0025% neutral red, 0.01% chrysoidin and 0.01% dahlia, as well as methylene violet + HCl stain the tissues of the former plants, but do not stain (dahlia and methylene violet + HCl) or stain slightly and irregularly (neutral red and chrysoidin) the tissues of the latter plants.

The staining reaction was found to change (Bassarskaja, 1936) also with further advance in development; thus, in *Triticum* × *Agropyrum* hybrids, the developing spikes recovered the faculty to stain blue and the epidermis of earing plants stained violet-red.

(5) Localization

Of cuttings taken from a soybean, all the plants derived from the sympodial shoots flowered much earlier than those from monopodial shoots (Lysenko, 1932e, 1935a). The situation on the stem alone does not yet predetermine the ease or time of flowering. Cuttings of a parent plant previously grown in continuous day rooted and grew rapidly, but did not flower in continuous day, whereas those from a plant grown previously in short day flowered rapidly. Again, in cotton which had overwintered in a greenhouse, monopodial shoots which grew in the spring remained sterile even when all the shoots were removed above the point of insertion of the first sympodium of the previous year. The inception of flowering depends, therefore, upon whether the cells of the formative tissues have undergone certain qualitative readjustments and seems to be independent of the upward current of nutrients.

Soybean cuttings from successive points along the stem flowered later the lower their situation on the stem, showing that the tissue may be "qualitatively" different along the stem and that the lower parts, although older, are less advanced in development than the relatively younger tissues above. By treating epidermal cells of leaves situated above and below the terminal bud with vital stains, Bassarskaja (1934a, b) confirmed this developmental difference, the upper leaves staining as reproductive, the lower as sterile. It may be inferred that while the plant or its parts traverse a developmental phase the internal changes occur only in the promeristem and are transmitted therefrom to daughter cells in which further qualitative changes take place, i.e. the movement of the acquired quality is effected only through cell division. To confirm this, Lysenko (1935a) quotes his observations that the sterile part of a plant cannot influence the fruiting part of the same plant and vice versa.

The strict localization of properties acquired by tissues limits the practical use of vernalization with polycarpic perennial plants, the stems arising from dormant buds in subsequent years requiring annual vernalization of the phase or phases which cannot be passed in the natural habitat. Čepikova (1935) reported what conventionally could be termed an "after-effect of vernalization", namely, all the plants vernalized in the previous year flowered earlier than the control in the following year. This can hardly be attributed directly to vernalization, as Lisicyn (1925) reported a similar after-effect in studying the time of flowering (earliness) of red clover in relation to manurial treatment. There is apparently a close connexion between the end of the first developmental cycle and the initiation, and thus

indirectly, the completion of the next cycle; winter dormancy does not appear to be a kind of demarcation, can be enforced at any time and is not voluntary.

(6) *Causal mechanism*

Garner and Allard in 1925 and later Razumov reported, however, that the inner changes may be formed in one part and transported to another. The question whether the ability to react to the thermal or photoperiodic effect is confined to special organs or, on the contrary, is inherent to all the parts of the plant has been the subject of many investigations.

McKinney (1935) attempted to locate the active region by decapitating the roots and coleoptile of wheat seedlings. Decapitated and intact seedlings, on being vernalized, headed at the same time. Therefore "the active region in question is not confined to the apical region of seminal roots, the coleoptile or the first leaf" and most probably the entire embryo is sensitive to temperature.

But this seems to differ in the case of photoperiodic response. In Litvinov's investigation (1934) of the active region, flowering was simultaneous, although during the first 28 days after planting the growing points of the experimental plants kept periodically under light-proof caps received 242 hr. additional darkness.

As early flowering and a shortened vegetative period, together with some correlative changes in height occurred only when leaves or leaves and stem were exposed to a short day, Psarev (1936) suggested that the functional sensitiveness to photoperiods is not confined to leaves alone, but is scattered over the whole body of the plant, although the leaves appear to be the principal agency. A connexion between the causal effect of light and the activity of leaves is evident also from the fact that the photoperiodic effect of light of varying quality is parallel with its absorption by leaves (Katunskii, 1937).

The functions of leaves are still more pronounced in the experiments of Čailahjan (1936, 1937) and Moškov (1935, 1937) with variously defoliated plants or with parts of plants grown in different photoperiods; the young upper leaves were either inactive or, at least, less active than the four or six leaves below; the lowest leaves also showed little activity; only the leaves of the central part of the stem were most active; the growing point was quite passive and the photoperiodic effect was expressed therein through the nearest of the active leaves. Such a localization of the region sensitive to light suggests (Litvinov, Čailahjan, Moškov) that under the photoperiodic effect certain catalytic substances are formed which are transmitted to the growing point, to participate there in morphogenesis. Moškov and Čailahjan suggest that this substance is in the nature of a hormone and have named it "florigen".

It is claimed (Moškov) that when the active leaves are exposed to photoperiods inhibiting reproduction, they secrete another substance with properties inhibiting flowering. The movement of either of these substances in the stem, mostly upwards to the nearest growing point, is thought to be regulated by the photosynthetic activity of the tip leaves. Thus a new version of an old idea of Sachs in 1865 has been revived.

Katunskiĭ (1936) formed a somewhat different opinion as to the significance of hormones, finding them to be inhibitory factors and stating that the photoperiodic effect consists in their destruction in the leaves.

The consideration of these hypotheses is, however, outside the scope of this review, as their experimental evidence rests on photoperiodic investigations. It should be pointed out, however, that the conclusion that florigen is not formed when active leaves are exposed to continuous darkness or continuous light and that the photoperiodic response is connected with the assimilative functions of leaves may be largely due to the shading of the plants throughout most of the vegetative period.

A different functional role is attributed to leaves by Razumov (1935), namely, through the apparent dependence of the rate of developmental processes upon the growth rate, which in its turn may be controlled by the environment acting upon the leaves. Two compensatory experiments with millet are quoted in support of this postulation. In the first, partially defoliated plants previously grown for 12 days in continuous day and high temperature flowered in an 8 hr. day 2-4 days later than the intact, i.e. the second stage had been insignificantly retarded. In the other experiment, plants were grown for 10 days in an 8 hr. or a 3 hr. day, supplemented by 10 lux illumination; in the first case flowering was 21 days earlier, i.e. the second phase was considerably retarded by impairing the growth rate consequent upon a reduction in sunlight from 8 to 3 hr. daily.

Recent investigations indicate, therefore, that the photoperiodic response of the growing point is not a direct result of light action, but is due to the formation of certain substances in the leaves, i.e. the changes which occur in the growing point are not primary but secondary. Whatever may be the nature of the substance formed in the leaves, their action is only of a catalytic nature. Otherwise, the developmental differentiation of the tissues along the stem would remain unexplained, while this difference is not less evident in the examples of induced sterility quoted by Razumov (1935) in which only the shoots arising near or from the sterile ears were reproductive.

There seems to be no doubt that the internal changes which are responsible for morphogenesis occur and are localized in the meristematic cells of the growing point. The fact alone that these changes can be attained through vernalization seems to suggest that the mechanism, but not the localization, of the causal changes is in question. While stating that for the completion of a phase an awakened embryo and a tillering plant would require exactly the same environmental conditions and would undergo exactly the same internal changes, Lysenko, of course, does not mean that the functional mechanism is necessarily the same.

VII. FURTHER ADVANCES IN THE THEORY OF PHASIC DEVELOPMENT

It has been shown that with the advance to reproduction a plant changes its internal environment, becoming, so to speak, changed qualitatively, and it is only natural that its relation to the habitat and hence its functions become different. Under the same light and temperature, for instance, CO₂ was less intensively

assimilated in vernalized plants (at the second phase) than in unvernallized plants (at the first phase), and the rate of assimilation increased rapidly in unvernallized plants, but remained practically unchanged in vernalized plants, when the day was prolonged from 9 to 24 hr. (Kuperman, 1935).

(1) *Genotype in development*

With advance in development some properties change, e.g. winter hardiness and the ability to be hardened in cereals increase with advance in the first phase, reach a climax at the end of this phase and fall with advance through the second phase and the parallel development of floral organs (Vasiljev, 1934*b*; Timofeeva, 1934*a*, 1935; Kuperman, 1934, 1935; Saltykovskiĭ, 1936; Šestakov, 1936, 1937). There is also a direct correlation between the length of the first phase and winter hardiness in wheat (Buřlina, 1935). Again, the length of the second phase, decreasing southwards, is in lucerne associated with a parallel change of rosette from prostrate to erect and a fall in winter hardiness (Koreřša, 1935). Drought resistance also changes with the advance in development, falling after tillering in most wheat varieties (Stefanovskiĭ, 1935).

These facts suggest that the internal readjustments during a phase are not merely responsible for its advance towards the ultimate goal, reproduction, but are also associated with vital functions and characters of the plant.

The inception of organs and characters is not only dependent upon the completion of a phase but their expression is to a great extent affected by the manner in which the phase was completed. In wheat (Kostjučenko, 1934) the 1000 grain weight was increased from 15.3–20.7 to 32.6–30.2 g. in unvernallized plants and in vernalized plants from 16.3–22.0 to 27.7–47.7 g., when the same varieties were grown in a longer day. Other characters also change, e.g. the spikes of the main stem may be awned, whereas those on other stems developing later are awnless (Lysenko, 1934). In experiments with the so-called "training of plants" a shortening of the first phase in winter cereals was accompanied by parallel changes in some morphological characters, such as awns, ears, glumes and leaves. When late and phylogenetically more ancient lucernes were grown in a shortened day the shape of the rosette was changed, whereas it remained unchanged in early races originating from the late (Sinskaja, 1937).

The vegetative period and its correlation with other characters have indeed been the subject of many genetical studies, but with rather divergent results and conclusions. In fact, no agreement could be reached, largely due to the fact that in these studies the vegetative period was dealt with as a whole, as a single character, while it consists of several consecutive, but mutually independent phases, of which at least the first two vary independently with ecotypes and may be recombined independently in the progeny, i.e. a short first phase in some plants may be associated with a long second phase and vice versa.

(2) *Genetical conceptions*

Faced with these facts Lysenko and his collaborators began genetical studies and were, as a matter of course, compelled to work upon somewhat modified genetical principles which are presented below in a condensed form without detailed survey, as the matter is still under active discussion.

The hereditary basis of a plant, argues Lysenko (1934, 1935*b*), is a fusion of two phylogenetical lines of parents; consequently it is more heterozygous and richer in potentialities than any of the parental forms. In order to realize these potentialities into developmental phases and the latter into organs and characters, the genotype requires appropriate conditions.

In the F_1 generation that allelomorph will develop which finds conditions appropriate or more favourable for its manifestation. Therefore, the dominance of a character rests upon the adaptability of the zygote in its biological development. In order to predict the dominance of the vegetative period, for example, it is necessary to know the type of the component phases, as well as the causal factors and the phase which induce the plants to develop into spring or winter, or late or early types, as the hybrid will develop along those phases which find the requisite conditions in the habitat. Hence a hybrid from winter \times spring or late \times early crosses, when sown in spring, will be of the spring or early type. In other conditions it may develop into a winter type.

Consequently, the F_1 plants from two late parents, one late because of a slow first phase, and the other because of retardation of the second, will under the same conditions be earlier than any of the parents, and no segregate in subsequent generations will be earlier than the F_1 plants or the segregates in the preceding generations. Hybridological studies with cereals and other plants of various biological groups have, it is claimed, fully substantiated this hypothesis.

(3) *Cytogenetical conceptions*

Therefore, according to this theory, plant development is controlled by the habitat within the limits of the hereditary basis of heterozygotes (genotype); subsequent investigations have shown, however, that the hereditary basis may be changed in ontogenesis by the environment (Lysenko, 1937). Some experiments with tomatoes and cereals and observations of field crops have compelled Lysenko to raise the question as to the desirability of revising certain cytogenetical concepts.

The entire germ cells, argues Lysenko (1934-7), and not only the genes localized in their chromosomes, are the physical basis of heredity; the germ cells, being an inseparable part of the organism, must bear and retain the impress of all those changes undergone, under the influence of the environment, in ontogenesis. As proof of this statement, Lysenko refers to the degeneration of pure lines of cereals and tomatoes as a result of perpetual self-pollination, when cultivated long enough on a large scale. The degeneration is caused through narrowing the range (potentiality) of adaptability of the genotype, as the male and female germ cells developing in the same floret or on the same plant are similarly modified by the habitat and thus contribute the same potentialities to the zygote. The de-

generation could be prevented by systematic intercrossing within the same line. In cross-pollination the gametes developing in a relatively different environment are modified in different ways and thus on fusion enrich the zygote. It is claimed that numerous experiments with intravarietal crosses carried out in various places on a large scale fully substantiate this claim.

Furthermore, Lysenko refers to experiments on altering the genotype of cereals in a desired direction by growing plants and their progeny in a specially selected environment; winter wheat is forced to reproduce at 15–20° C. in place of the optimal 0–3° C.

Vernalized cells, states Lysenko (1937), are localized in the growing point and from them the uninterrupted chain of cells of straw and ear, and the male and female germ cells are later developed. The vernalized cells are thus the basic cells for the further construction of the entire plant body which eventually produces the seeds. Hence, if the vernalized cells of two plants of the same line become different owing to a variation in conditions during the first phase (vernalization), this difference will be passed on to the germ cells and transmitted into the new seeds (progeny). This method of altering the genotype of the plant has been termed "training". It is claimed that with its use the I_3 of winter plants behaved as typical spring forms and that this conversion of winter forms into spring forms was gradual and cumulative and associated with changes in some morphological characters. Similarly, it is claimed, spring cereals were converted into winter forms by forcing them to complete the first phase at temperatures much below the optimum.

The concepts announced by Lysenko since 1934 have been the subject of rather heated discussion which reached its climax at the Fourth Session held at the Academy of Agricultural Science in Moscow, December, 1936. No detailed survey of the question is here attempted, as the data available are still incomplete and the matter is in a rather controversial state. It should be stated, however, that the idea that Lysenko is opposed to cytogenetics is an undue exaggeration, as there is a difference between a modification of some genetical conceptions, even if they may be fundamental, and the destruction of the entire science. In fact in some questions, such as the relation and dependence of dominance and the manifestation of the genotype in general upon the environment there is little discrepancy between orthodox genetics and Lysenko's hypothesis. As regards the mechanism of inheritance, Lysenko, while maintaining that the entire cell participates in the maturation division, means that it does so "functionally". Mendelian inheritance on a chromosomal basis does not exclude other forms of inheritance depending upon other cell material, or the effect of protoplasm on inheritance as, for instance, when multiplication of the chromosomes is not accompanied by a parallel increase in the volume of cytoplasm. In the light of recent investigations the gene does not appear to be as stable as was previously thought, while the very nature of gene stability (or is it a variation about a definite mode?) is still obscure. On the other hand, "training of plants" has not been investigated cytologically, nor is there any evidence that the "trained" plants were tested under normal conditions.

As regards the pure line conception, "where only a few plants are grown annually

under controlled conditions the chances are great that many years would elapse before the single mutation would be observed", but it is different when a pure line is represented by millions of individuals; "the number of mutants, in spite of their comparative rarity, are sufficiently numerous to be readily detected... I venture to think that the progeny of any one of Johanssen's isolated 'pure lines' would become a similar complex mixture of lines exhibiting hereditary different characters if propagated long enough on a large scale" (Percival, 1934). The term pure line is genological and relative, based upon morphological characters; heterozygosity in some physiological characters, such as respiration energy (Dix, 1931) or suction force (Buchinger, 1936), is not excluded and, as Baranskiĭ (1936), Razumov (1937) and Kiričenko (1937) have shown, pure lines may be split up into a number of biological populations in respect of their photoperiodic response.

VIII. SUMMARY

1. Investigations on the cause of the failure of winter plants to ear in the sowing year have led to a theory of plant development based upon a discrimination between growth and development. The latter is regarded as a sequence of qualitative changes or phases, each consisting of complex internal readjustments which lead to eventual reproduction. The developmental phases proceed in a strict rotation and a subsequent phase cannot begin until the preceding phase has been completed.

2. Although growth has no causal connexion with development, the true relationship between development and growth has not been conclusively investigated, while some circumstantial evidence suggests that development can be maintained only with a certain minimum of growth.

3. As development is at least relatively independent of growth, the embryo can be induced to develop at a much reduced growth rate before sowing and even before complete seed ripeness. This is known as vernalization. Although vernalization has no direct effect on the vegetative period after sowing, subsequent development proceeds under relatively different conditions. The effect of vernalization is thus analogous to that of a change in time of sowing.

4. The first three developmental phases and a possible transitional phase between the first two have already been established; the first two and the transitional phase being connected with the initiation of floral organs and the third with gametogenesis.

5. For completion of each of the developmental phases a different but definite complex of environmental factors is required. It is essential to discriminate between factors which do or do not affect the progress of a phase. In addition, among factors which affect progress, those which are indispensable for a phase must be distinguished from those which affect the progress of a phase only in the presence of indispensable factors.

6. For vernalization of the first phase a definite balanced complex of temperature, moisture and aeration is required by a physiologically potent embryo. This complex varies widely within and between species. The effect of light and darkness has not been convincingly demonstrated, although the indirect effect of day length

is not excluded, particularly for a plant which is distinct from the embryo in a seed as regards the mode of nutrition. A critical thermoperiod can be established, below which the vernalizing temperature becomes ineffective.

7. The complex of factors required for the second and subsequent phases has not been fully studied. In environmental studies particular attention must be given to the optimal blend of day length and temperature. Long-day plants require light to complete the second phase and can only tolerate darkness if the latter is not in excess, while short-day plants show the opposite relation. The darkness requirement of short-day plants can be deciphered as an inhibitory effect of light upon the second phase, if the intensity of light is above a definite maximum. The relation of cereals at the third phase seems to be in conflict with Lysenko's ecological concepts.

8. The physiology of plant development has received little study. Not all the changes pertaining to development are detectable morphologically. The changes constituting a phase are gradual, quantitative, additive and irreversible, and their completion causes a qualitative change in the internal environment of a plant. Thereby the properties and relation of a plant to its environment are also changed.

9. The changes pertaining to an advance in development are elaborated and retained in the promeristem and transmitted only through cell division, further elaboration taking place in the daughter cells. The entire body of the embryo is sensitive to temperature, whereas the response of the promeristem to photoperiods is effected through some activities in the leaves. In the latter a catalytic substance is secreted, which on being transmitted to the tip participates in the developmental processes.

10. The nature of the changes constituting the first phase has received little attention. The hormonal theory of vernalization is inconsistent with the theory of phasic development and has not been substantiated. Of the enzymes studied, only catalases and peroxidases were suggestive. Presumably the enzymes and hormones are concerned more closely with the rate of growth than of development. The endosperm, aleurone layers or integuments do not participate directly in the developmental process, which is confined to the embryo alone. Investigations of the physical and chemical changes in the protoplasm in relation to phasic development were more suggestive and provided a method of diagnosis of the first two phases. The nature of the second and subsequent phases has not been studied.

11. In environmental studies the biochemical method of diagnosis should be employed whenever possible, as the conceptions based upon the "after-effect of vernalization" are not always reliable. The possibility of vernalizing the embryo during seed ripening has necessitated a thorough revision of the conceptions hitherto formed regarding the length of developmental phases and earliness or lateness in various ecotypes.

12. The genetical conceptions announced by Lysenko differ but little from the orthodox conceptions, greater stress having been laid on the effect of the environment. The cytogenetical conceptions require further study, while the changes in the genotype of a plant induced, as it is claimed, as a result of adaptation to a new environment (training of plants) must be studied cytologically.

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ADDENDUM

E. Larose and R. Vanderwalle (*Bull. Inst. agron. Gembloux*, 7, 149-62, 1938) present results of experiments on wheat which seem to support the view stated at the conclusion of Part V (p. 71). When the plants from vernalized or unvernallized seeds were grown, first in a 9-hour day for 1, 2 or 3 months and then in a long day, earing was retarded, as compared with plants grown in a long day throughout; in some cases, however, the longer duration of shading seemed to speed up development as compared with the shorter durations. This is particularly evident with Jubilé winter wheat; the vernalized plants eared in the long-day series on June 28, in the one month series on July 10, in the two month series on July 23, and in the three month series on July 22. The unvernallized plants eared on July 15 and August 20, 24 and 20 respectively. The experiments were not suitably arranged to detect this "accelerating" effect of the third and in some instances of the second month of shading in all cases. It might, however, almost seem that in all cases the "retardive" effect of the second and particularly of the third month was less potent than that of the first month. This is more evident with mid-season strains and, whenever vernalization was effective, with unvernallized plants. This regularity cannot be fully ascribed to the gradually decreasing difference between the nine-hour day and the length of the seasonal day concurrent with and subsequent to the shading period, which was, of course, another fault in the arrangement of these experiments.

KEIMBLATTCHIMÄRENFORSCHUNG AN SEEIGELLARVEN

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(Received 19 May 1938)

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I. EINLEITUNG

Vor 5 Jahren habe ich in zwei zusammenfassenden Arbeiten (1933c, 1934a) über die damals vorliegenden Ergebnisse der Keimblattchimärenforschung an Seeigellarven berichtet. Inzwischen ist diese Forschung in verschiedenen Richtungen weiter getrieben worden und es konnten neue Ergebnisse erzielt werden.

Die genannten Keimblattchimären wurden in der Weise hergestellt, dass die Mikromeren des 16-Zellenstadiums eines Seeigelkeimes in die Blastula eines anderen Keimes implantiert wurden. Die Blastula kann entweder aus einem Ganzkeim hervorgegangen sein oder aus einem Fragmentkeim, der entweder das gesammte virtuelle Ektoderm (an_1 , an_2 , veg_1 , Hörstadius, 1931, 1935; v. Ubisch, 1933) oder nur 2/3 desselben (an_1 , an_2) umfasst. Da die Mikromeren das Skelettbildnermaterial enthalten, so besitzen die aus einem Ganzkeim + implantierten Mikromeren hervorgegangenen Keime ausser allen virtuellen Organbezirken des Wirtskeimes noch ein zusätzliches, vom Spender stammendes Skelettbildnersortiment, die aus einem Fragmentkeim hervorgegangenen Keimblattchimären ausser virtuellem Ektoderm des Wirts Skelettbildnermaterial ausschliesslich vom Spender. Letztere Keimblattchimären wurden zur Unterscheidung von den ersten als "reine Keimblattchimären" bezeichnet.

Die Keimblattchimären können weiterhin durch Implantation artgleicher Mikromeren erzeugt sein oder aber es werden die Mikromeren einer Art A in den Wirtskeim einer Art B implantiert. Je nach dem Verwandtschaftsgrad und der Struktur der Skelette können wir von homoioplastischer, skelettgleicher (*Psammechinus miliaris*-Wirt + *P. miliaris*-Spender, *P. microtuberculatus*-Wirt + *P. microtuberculatus*-Spender), heteroplastischer, skelettgleicher (*P. microtuberculatus*-Wirt + *Paracentrotus lividus*-Spender und reziprok), heteroplastischer, skelettungleicher (*Psammechinus microtuberculatus*-Wirt + *Sphærechinus granularis*-Spender), xenoplastischer, skelettungleicher (*Psammechinus miliaris*-Wirt + *Echinocyamus pusillus*-Spender und reziprok, *Psammechinus microtuberculatus*-Wirt + *Echinocardium cordatum*-Spender und reziprok) Kombination sprechen. Das normale Skelett von *Psammechinus miliaris* und *P. microtuberculatus* sowie *Paracentrotus lividus* zeigt Abb. 13, das von *Echinocyamus pusillus* Abb. 15. Das normale Skelett von *Sphærechinus granularis* ist in allen wesentlichen Punkten mit dem von *Echinocyamus pusillus* übereinstimmend. Dasselbe gilt für das Skelett von *Echinocardium cordatum*, nur besitzt diese Form ausserdem noch den gegitterten unpaaren Scheitelstab, wie ihn Abb. 4 zeigt.

II. REINE KEIMBLATTCHIMÄREN

1933c konnte über das Ergebnis der Kombination *Psammechinus miliaris*-animale Hälfte (an_1 und an_2) + *Echinocyamus pusillus*-Mikromeren und reziprok berichtet werden. Die erstere Kombination lieferte lediglich Blastulæ mit unvollständigen aber deutlich Gitterstabsnatur (Spender) aufweisenden Skeletten. Die reziproke ergab tetraederförmige Larven mit einfachen Skelettstäben. Es zeigte sich also: dass (1) die Skelettbildner im ordnungsfremden Wirt herkunftsgemässe Skelette aufbauen, (2) dass die Skelettbildner sich an den Stellen des Wirts anordnen, an denen auch die Wirtsskelettbildner normalerweise liegen und dass also (3) die ektodermalen Anlockungsfaktoren unspezifischer Natur sein müssen.

Da die genannten Larven ihre Entwicklung auf einem relativ frühen Stadium eingestellt hatten, so konnte unter anderem die Frage nicht entschieden werden, ob das ordnungsfremde Skelett imstande ist seine Rolle bei der weiteren Formgestaltung der Larve zu spielen, insbesondere die Ausgestaltung normal gebildeter Fortsätze zu fördern und diese mit entsprechenden Skelettstäben zu versehen. Andererseits hatte die Implantation artgleicher Mikromeren in 1/2- oder 3/4-Keime von *Echinocyamus* ganz normal gestaltete Plutei geliefert, denen nur die entodermalen Teile des Darmtrakts fehlten (1933, 1933c). Die Operation an sich konnte also nicht an dem unvollkommenen Ausfall des obigen Experimentes Schuld sein. Es wurden daher (1936) erneut die Mikromeren von *Echinocyamus* in animale Halbkeime von *Psammechinus miliaris* und reziprok implantiert und es konnte nunmehr ein klares Ergebnis erzielt werden. Abbildung 1 zeigt die beste Larve der ersten Kombination. Es ist nicht nur ein deutlich spendergemässes Skelett mit Gitterstab entstanden sondern auch ein typischer Analfortsatz mit Wimper-schnur. Abbildung 2 zeigt eine Larve der reziproken Kombination. Es finden sich

nur einfache, spendergemässe Skelettstäbe und wiederum ein wohlausgebildeter Analfortsatz. Beiden Larven fehlt natürlich der Darmtraktus, dessen virtuelles Material (*veg₂*) ja entfernt war, mit Ausnahme des Mundes, der ektodermaler Herkunft ist. Diese Versuche bestätigen also nicht nur das frühere Ergebnis, dass sich die Skelette strukturell herkunftsgemäss entwickeln und im Wirt ortsgemäss anordnen sondern zeigen, dass die Analstäbe auch zur Bildung ausdifferenzierter Fortsätze beitragen können. Die hierzu erforderlichen Reize müssen also unspezifischer Natur sein.

Inzwischen hat auch Hörstadius reine Keimblattchimären hergestellt, von denen uns hier nur die interessieren, bei denen Formen mit Gitterstäben mit solchen mit einfachen Stäben kombiniert wurden. Die Kombination: *Echinocardium cordatum*-gesamntes Ektoderm (*an₁*, *an₂*, *veg₁*) + *Paracentrotus lividus*-Mikromeren ergab anscheinend rein spendergemässe Skelette, wie nach obigem zu er-

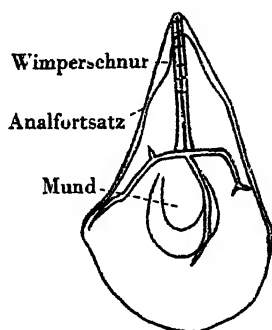


Abbildung 1.

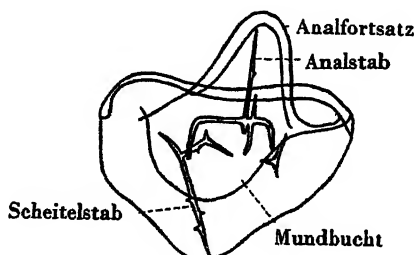


Abbildung 2.

Abbildung 1. "Reine" Keimblattchimäre *Psammechinus miliaris*-animale Halbblastula + *Echinocyamus pusillus*-Mikromeren. Entstehung eines spendermässigen gegitterten Analstabes und mehrerer Scheitelstäbe. Der Wirt bildet einen Analfortsatz mit Wimperschnur aus.

Abbildung 2. "Reine" Keimblattchimäre *Echinocyamus pusillus*-animale Halbblastula + *Psammechinus miliaris*-Mikromeren. Entstehung spendermässiger einfacher Stäbe. Der Wirt bildet einen Analfortsatz mit Wimperschnur aus.

warten war. Jedenfalls entstanden keine Gitterstäbe (Hörstadius, 1936, S. 863). Die Kombination: *Psammechinus microtuberculatus*-gesamntes Ektoderm + *Sphärechinus granularis*-Mikromeren ergab eine Larve mit intermediärem Skelett. Der wirtsgemäss keulenförmige anale Scheitelstab der einen Seite lässt keinen Zweifel, dass in diesem Falle vom Wirt regulativ Skelettbildner geliefert worden sind und wir es also in Wirklichkeit nicht mit einer reinen Keimblattchimäre zu tun haben, wie auch Hörstadius selbst erwägt (S. 864-5). Das Auftreten von Brücken bei dieser Kombination ist ein neues Ergebnis, das ich bereits 1932 (S. 64) in Aussicht gestellt habe. Der Versuch müsste zweckmässiger Weise so abgeändert werden, dass man als Wirt nicht $3/4$ sondern nur animale $1/2$ -Keime von *Psammechinus* nimmt, um die Gefahr regulativen Ersatzes der Wirtsskelettbildner zu vermeiden. Die Spendermikromeren müssten nicht wie bei Hörstadius transplantiert sondern in das Blastocöl der Wirtshalbblastula implantiert werden, um unkontrollierbaren Induktionswirkungen derselben zu entgehen.

III. XENOPLASTISCHE, SKELETTUNGLEICHE KEIMBLATTCHIMÄREN

Die xenoplastische Kombination: *Psammechinus miliaris*-Ganzkeim + *Echinocyamus pusillus*-Mikromeren und reziprok hatte Keimblattchimären mit intermediärem Skelett ergeben (1931a, 1933a, 1933c). Dass die reziproken Kombinationen hinsichtlich des Skeletts—etwa im Gegensatz zu reziproken Bastardkom-

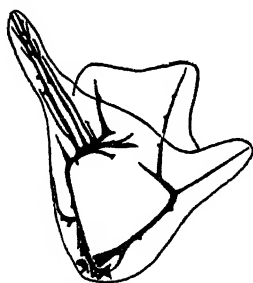


Abbildung 3.

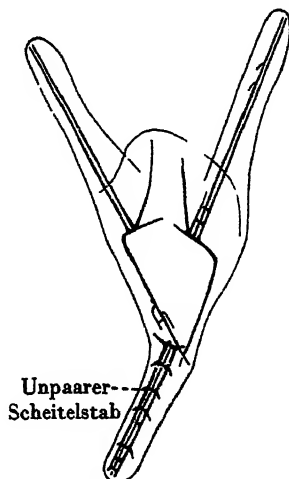

 Unpaarer
Scheitelstab

Abbildung 4.



Abbildung 5.

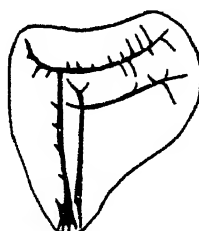


Abbildung 6.

Abbildung 3. Keimblattchimäre *Psammechinus microtuberculatus*-Ganzkeim + *Echinocardium cordatum*-Mikromeren. Das linksseitige Skelett intermediär.

Abbildung 4. Keimblattchimäre *Echinocardium cordatum*-Ganzkeim + *Psammechinus microtuberculatus*-Mikromeren. Analstabskelett beiderseitig intermediär. Der unpaare Scheitelstab rein wirtsgemäss.

Abbildung 5. Bastard *Echinocardium cordatum* ♀ × *Psammechinus microtuberculatus* ♂. Skelett intermediär. Der unpaare Scheitelstab fehlt.

Abbildung 6. Bastard (?) *Psammechinus microtuberculatus* ♀ × *Echinocardium cordatum* ♂. Aus Altrogge (1935), Abbildung 34a.

binationen—übereinstimmende Resultate zeitigen, ist verständlich, nachdem wir einmal festgestellt haben, dass sich die implantierten Mikromeren im ordnungsfremden Wirt herkunftsgemäss verhalten und zweitens ja bei beiden Kombinationen je ein *Psammechinus*- und ein *Echinocyamus*-Skelettbildnersortiment vereinigt wurden. Für die Kombination: *Psammechinus microtuberculatus* + *Echino-*

cardium cordatum (1931a, 1932) lag zur Zeit des Berichtes von 1933c nur die eine Kombination: *Psammechinus microtuberculatus*-Ganzkeim + *Echinocardium cordatum*-Mikromeren vor, die ebenfalls intermediäre Skelette ergeben hatte. Es war aber in diesem Falle besonders wünschenswert auch die reziproke Kombination herzustellen, da ja die beiden genannten Formen sich nicht nur hinsichtlich der Struktur ihrer Skelette (Gitterstäbe—einfache Stäbe) unterscheiden, sondern *Echinocardium* in dem unpaaren Scheitelstab (Abbildung 4) ein Skelettstück besitzt, das *Psammechinus* überhaupt fehlt. Bei der oben genannten Kombination war es niemals aufgetreten sondern bestenfalls ein im Scheitel gelegener Skelettstab, der möglicherweise dem hinteren Querstab von *Echinocardium*, der die Basis des unpaaren Scheitelstabes bildet, entspricht. Ebensowenig trat jemals ein unpaarer Scheitelfortsatz auf. Es wurde daher die Kombination: *Echinocardium cordatum*-Ganzkeim + *Psammechinus microtuberculatus*-Mikromeren ausgeführt und über das Ergebnis von mir (1934) und Schmidt (1936) berichtet.

Wiederum ergaben sich wie bei der reziproken Kombination (Abbildung 3) intermediäre Skelette (Abbildung 4), und es wurde damit das Ergebnis bei den entsprechenden reziproken Kombinationen von *Psammechinus miliaris* und *Echinocyamus pusillus* bestätigt. Aber die Keime wiesen nunmehr die Eigentümlichkeit auf, dass der unpaare Scheitelfortsatz nebst unpaarem Scheitelstab vorhanden sein konnte und zwar im Gegensatz zu dem übrigen Skelett stets in rein wirtsgemässer, nie in intermediärer Gestaltung. Dies zunächst überraschende Ergebnis wird verständlich, wenn wir uns daran erinnern, dass der Spender *Psammechinus* keinen unpaaren Scheitelstab besitzt und wir demzufolge annehmen dürfen, dass die implantierten *Psammechinus*-Skelettbildner an der Bildung dieses Skelettstückes nicht teilnehmen. Wir werden weiter unten hierauf zurückkommen.

IV. BASTARDE

1931a wurde auf die Ähnlichkeit der Keimblattchimären mit den Bastarden entsprechender Kombination hingewiesen und die cytologischen Grundlagen dieser Übereinstimmung erörtert (1933a, 1933c). Es lag damals nur die Kombination: *Echinocardium cordatum* ♀ × *Psammechinus miliaris* ♂ (Abbildung 5) vor. Der intermediäre Charakter des Skeletts ist deutlich und es ist von besonderem Interesse, dass allen Bastarden der unpaare Scheitelstab, der der Mutter zukommt, fehlt. Inzwischen ist es uns geglückt 1934 in Neapel auch die reziproke Bastardkombination zur Entwicklung zu bringen, worüber Altrogge (1935) berichtet hat. Leider konnten die Larven nicht sehr weit herangezüchtet werden (Abbildung 6) und sind so stark pathologisch, dass trotz gewisser intermediärer Eigenschaften, da der cytologische Nachweis der Diploidie nicht erbracht ist, der Verdacht besteht, es könnte sich um experimentell erzeugte Parthenogenese handeln. Sollte es sich doch um Bastarde handeln, so wäre das Fehlen des unpaaren Scheitelstabes von Interesse.

V. STOCKWERKCHIMÄREN

Schmidt (1936) hat über Versuche berichtet, bei denen Keimen von *Echinocardium cordatum* im 16-Zellenstadium die vier Mikromeren abpräpariert und dann dem Restkeim im Blastulastadium die Mikromeren von *Psammechinus microtuberculatus* implantiert wurden. Nun wissen wir, dass nach Amputation der Mikromeren die Restkeime trotzdem Skelett entwickeln können, indem von dem *veg₂*-Material regulativ Skelettbildner gestellt werden (Literatur siehe bei Schmidt). Aber diese Regulation braucht einen gewissen Zeitraum zu ihrer Vollendung (v. Ubisch, 1934, S. 102) und in den oben erwähnten Keimen konnten sich also zunächst die Implantatskelettbildner ungehindert auswirken und erst nachträglich

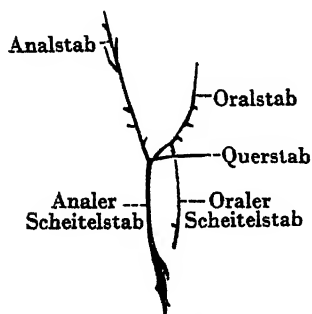


Abbildung 7.

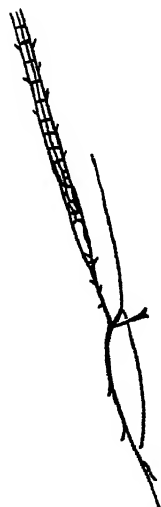


Abbildung 8.

Abbildung 7. Stockwerkchimäre *Echinocardium cordatum*-Wirt ohne eigene Mikromeren + *Psammechinus microtuberculatus*-Mikromeren. Überwiegend spendermässiges Skelett. Man beachte den keulenförmigen Scheitelstab und den einfachen Analstab. Aus Schmidt (1936), Abbildung 35a.

Abbildung 8. Dieselbe Stockwerkchimäre wie in Abbildung 7 fünf Tage später bei gleicher Vergrößerung. Das Skelett ist nunmehr überwiegend wirtsgemäss. Die spendermässige Keule des Scheitelstabes ist rückgebildet, der Analstab basal spendergemäss, in einer Zwischenzone intermediär, distal wirtsgemäss. Aus Schmidt (1936), Abbildung 35c.

traten die Wirtsskelettbildner in Funktion. Dementsprechend konnten die Keime anfangs einen überwiegend spendermässigen (*Psammechinus*) Charakter besitzen (Abbildung 7). Insbesondere ist der keulenförmige Scheitelstab und der einfache Analstab zu beachten. Fünf Tage später jedoch war die Keule des Scheitelstabes rückgebildet und ein typischer *Echinocardium*-Scheitelstab entstanden. Der Analstab verwandelte sich über eine Zwischenzone in einen immer vollkommeneren *Echinocardium*-Gitterstab (Abbildung 8). Wir sehen hier also die gleichzeitige Bautätigkeit der beiden Skelettbildnersortimente bei den gewöhnlichen Keimblattchimären in eine zeitlich aufeinander folgende bei den "Stockwerkchimären" zerlegt. Hierher gehört sinngemäss auch die im II. Abschnitt erwähnte, von

Hörstadius hergestellte Chimäre: *Psammechinus microtuberculatus*-gesamntes Ekto-derm — *Sphärechinus granularis*-Mikromeren, die sowohl in den Analstäben wie auch im analen Scheitelstab der dem Beschauer zugekehrten Seite deutlich Stockwerknatur aufweist (Hörstadius, 1936. Abbildung 28).

VI. BASTARDCHIMÄREN

Wie 1933c ausgeführt, besteht hinsichtlich der Skelettbildung der wesentliche Unterschied zwischen den Keimblattchimären und Bastarden darin, dass die ersteren in ihren Skelettbildnern Plasma beider Ausgangsformen, die Bastarde dagegen nur mütterliches Plasma besitzen. Eine vergleichende Analyse beider Kombinationen würde wesentlich gefördert werden, wenn es möglich wäre einplasmatische Keimblattchimären herzustellen. Dies wurde in der Weise erreicht, dass den Wirtskeimen der Art A Mikromeren implantiert wurden, die einem Bastardkeim der Kombination: $A \varnothing \times B \sigma$ entstammten. Diese "Bastardchimären" besitzen dann sowohl in den Wirts- wie in den Spendermikromeren nur Plasma der Art A, ausserdem aber in den Wirtsmikromeren 100% A-Chromatin, in den Spendermikromeren 50% A- und 50% B-Chromatin. Das Ergebnis solcher Bastardchimärenkombinationen ist von mir (1934) und Altrogge (1935) beschrieben worden. Abbildung 9 zeigt die Kombination: *Echinocardium cordatum*-Wirt + *Echinocardium cordatum* $\varnothing \times$ *Psammechinus microtuberculatus* σ -Mikromeren, Abbildung 10 die reziproke Kombination: *Psammechinus microtuberculatus*-Wirt + *Psammechinus microtuberculatus* $\varnothing \times$ *Echinocardium cordatum* σ -Mikromeren. Es ist offensichtlich, dass die beiden Bastardchimären zwar intermediären aber überwiegend Wirts-respektive mütterlichen Charakter aufweisen. Das kann nicht auf dem Plasmagehalt der Skelettbildner beruhen, denn dieser stimmt mit dem gewöhnlichen Bastarde überein, die, wie wir oben (Abbildung 5) sahen, viel stärker intermediär sind. Es liegt im Gegenteil nahe die stark wirtsgemässe respektive mütterliche Prägung auf die Tatsache zurückzuführen, dass für die Skelettbildung $3/4$ Wirts-respektive mütterliches und nur $1/4$ Spender-respektive väterliches Chromatin zur Verfügung standen. Wir können dies aber in der Weise prüfen, dass wir, wie im Abschnitt V dargelegt, den Wirten vor der Implantation der Bastardmikromeren 1 oder mehrere Mikromeren wegschneiden. Dementsprechend verschiebt sich dann das Chromatinverhältnis zu Gunsten des Spenders respektive Vaters. Abbildung 11 zeigt eine solche Bastardchimäre der Kombination: *Echinocardium cordatum*-Wirt weniger drei eigene Mikromeren + *Echinocardium cordatum* $\varnothing \times$ *Psammechinus microtuberculatus* σ -Mikromeren. Abbildung 12 gibt eine Bastardchimäre derselben Kombination wieder, jedoch waren in diesem Falle alle vier Mikromeren abpräpariert worden. Schon Abbildung 11 zeigt gegenüber Abbildung 9 ein wesentlich mehr spender-respektive vätermässiges Aussehen. Abbildung 12 nähert sich dem Aussehen eines Bastards—insbesondere fehlt der unpaare Scheitelstab—ist aber doch in Anbetracht der verhältnismässigen Regelmässigkeit der Brücken wohl als Chimäre zu werten, das heisst, es dürften auch hier nachträglich regulativ entstandene Wirtsskelettbildner in Funktion getreten

sein. Die theoretische Bedeutung des Versuchs besteht darin, dass er ermöglicht "die bedeutungsvolle Frage der Auswirkung verschiedener Chromatinquantitäten in den Bereich der Untersuchung zu ziehen" (Altrogge, 1935, S. 270). Es zeigt sich, dass es durch Abstufung der Chromatinproportion der beiden ordnungsverschiedenen Partner möglich ist "den Phänotypus des Skeletts vom interme-

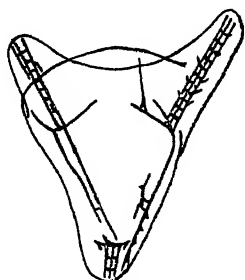


Abbildung 9.

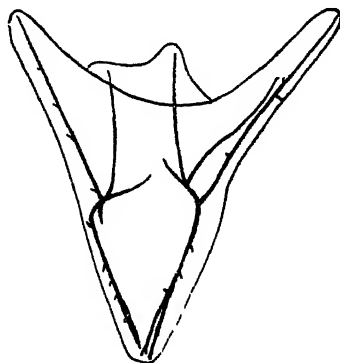


Abbildung 10.

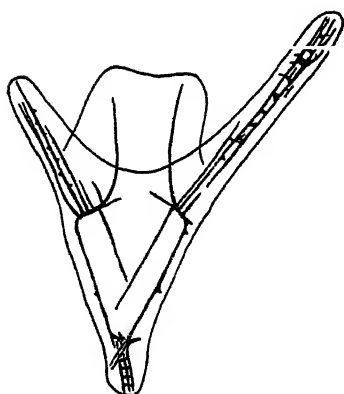


Abbildung 11.

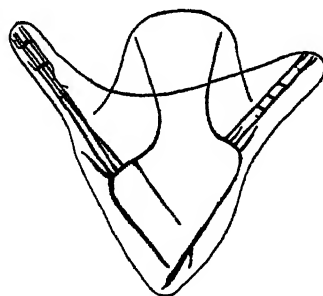


Abbildung 12.

Abbildung 9. Bastardchimäre *Echinocardium cordatum*-Wirt + *Echinocardium cordatum* ♀ × *Psammechinus microtuberculatus* ♂-Mikromeren. Skelett überwiegend wirtsgemäss respektive mütterlich.

Abbildung 10. Bastardchimäre *Psammechinus microtuberculatus*-Wirt + *Psammechinus microtuberculatus* ♀ × *Echinocardium cordatum* ♂-Mikromeren. Skelett überwiegend wirtsgemäss respektive mütterlich.

Abbildung 11. Bastardchimäre *Echinocardium cordatum*-Wirt weniger 3 eigene Mikromeren + *Echinocardium cordatum* ♀ × *Psammechinus microtuberculatus* ♂-Mikromeren. Skelett stärker intermediär als in Abbildung 9.

Abbildung 12. Bastardchimäre *Echinocardium cordatum*-Wirt weniger 4 eigene Mikromeren + *Echinocardium cordatum* ♀ × *Psammechinus microtuberculatus* ♂-Mikromeren. Skelett intermediär. Der unpaare Scheitelstab fehlt.

diären Typus zu weitgehender Dominanz der einen Ausgangsform zu verschieben" (v. Ubisch, 1934, S. 103). Die reziproke Kombination ergab zwar gleichsinnige, aber weniger präzise Resultate, was Altrogge wohl mit Recht auf die Hinfälligkeit (siehe Abschnitt IV) der als Spender verwandten Bastardkombination zurückführt.

VII. ANALYSE DER ENTSTEHUNGSBEDINGUNGEN DES UNPAAREN SCHEITELSTABES VON *ECHINOCARDIUM*

Der Vergleich von Bastarden, Keimblatt- und Bastardchimären erlaubt in einem Spezialfall, nämlich betreffs der Bildung des unpaaren Scheitelstabes von *Echinocardium* eine Analyse der für seine Entstehung massgebenden Faktoren einzuleiten. Dies ist deshalb von Bedeutung, weil es einerseits weder erhofft werden kann noch sich als lohnend erweisen dürfte die zu einer in der üblichen Weise durchgeführten genetischen Analyse erforderlichen weiteren Filialgenerationen der Bastarde zu züchten. Andererseits handelt es sich, soweit ich sehen kann, um den ersten Fall, in dem eine Faktorenanalyse ordnungsverschiedener Merkmale bei Bastarden angebahnt werden kann. Das Entstehen des unpaaren Scheitelstabes ist abhängig von zwei Faktorenpaaren: (1) Von dem Vorhandensein des virtuellen Fortsatzektoderms, (2) vom Vorhandensein der betreffenden virtuellen Skelettbildner. Bastarde besitzen keinen unpaaren Scheitelstab. Es muss also entweder das Fehlen des Fortsatzektoderms oder der Skelettbildner oder beider dominant über ihr Vorhandensein sein. Fehlt das virtuelle Fortsatzektoderm ganz, so können trotz Vorhandensein von *Echinocardium*-Mikromeren (Kombination: *Psammechinus*-Wirt + *Echinocardium*-Mikromeren, Abbildung 3) keine unpaaren Scheitelstäbe angelegt werden. Bei den Bastardchimären der Kombination: *Echinocardium* (ohne eigene Mikromeren) + *Echinocardium* ♀ × *Psammechinus* ♂ (Abbildung 12) ist das virtuelle Fortsatzektoderm vorhanden, trotzdem fehlt der unpaare Scheitelstab. Es muss also in den Bastardmikromeren die negative (*Psammechinus*)-Komponente dominant sein über die positive (*Echinocardium*)-Komponente. Über das Dominanzverhältnis innerhalb des Ektoderms würden wir durch folgenden Versuch Aufschluss erhalten können: Bastarden *Echinocardium* ♀ × *Psammechinus* ♂ werden die eigenen Mikromeren abpräpariert und die Mikromeren reiner *Echinocardien* implantiert. Bei Dominanz des *Echinocardium*-Ektoderms müsste nun der Scheitelstab gebildet werden, bei Rezessivität seine Entstehung unterbleiben. Der Versuch wurde unternommen, blieb aber leider bisher infolge der Hinfälligkeit des benutzten Bastardmaterials ohne Ergebnis.

VIII. DIE CYTOLOGISCHEN VORGÄNGE BEI DER NORMALEN SKELETTBILDUNG

Während die intermediäre Beschaffenheit der Bastardskelette auf der Heterozygotie des Skelettbildnermaterials beruhen muss, sind für die Deutung der Intermediarität der Keimblattchimärenskelette zwei Möglichkeiten vorhanden: Es ist denkbar, dass diese so zustande kommt, dass die ordnungsverschiedenen Skelettbildner ihrer Herkunft entsprechende Skelettabschnitte ausscheiden und so ein mehr oder minder feines Mosaik zustande kommt, das dann nur in seiner Gesamtheit einen intermediären Eindruck macht. Oder aber die Intermediarität ist wirklich das Ergebnis einer Mischwirkung, die von den ordnungsverschiedenen in einem gemeinsamen Plasmasyngyrium liegenden Kernen ausgeht. Im letzten Falle würde es sich um ein "genetisches Problem" (v. Ubisch, 1933a, S. 81)

handeln. Hörstadius (1936) hält beide Erklärungsmöglichkeiten für etwa gleichwertig, meint aber, dass die Mosaikhypothese "ausreichend" sein könnte (S. 868). Schmidt (1936) weist besonders darauf hin, dass eine genauere Kenntnis der Vorgänge bei der normalen Entwicklung, als wir sie bisher besaßen, erforderlich sei. Gegen die Mosaikhypothese sprechen neben einer ganzen Reihe von Befunden besonders die oben erwähnten Stockwerkchimären, welche ja sehr deutlich zeigen, wie nach einander an demselben Skelettstück herkunftsverschiedene Skelettbildner arbeiten können und die in den Übergangszonen Skelettabschnitte besitzen, welche man unmöglich als Mosaik deuten kann. Um sicher zu gehen, habe ich die normale Entwicklung des Skeletts einer Form mit Gitterstäben (*Echinocyamus pusillus*) und einer solchen mit einfachen Stäben (*Psammechinus miliaris*) untersucht (1937). Die uns hier interessierenden wichtigsten Resultate sind folgende:

(1) Die von Prenant (1926) und anderen Forschern aufgestellte Behauptung, dass die Skelettstäbe in einem vom Plasma der Skelettbildner eingegangenen Synzytium ausgeschieden werden, konnte vollauf bestätigt werden.

(2) Die einzelnen Zellkomponenten des Synzytiums führen, wie Lageveränderungen der Kerne und des sie umgebenden Entoplasmas erkennen lassen, wesentliche Wanderungen aus. Die Folge ist, dass der weitere Ausbau der Skeletteile, insbesondere ihr Dickenwachstum, von ganz anderen Skelettbildnern durchgeführt werden kann als denen, die den betreffenden Skelettabschnitt ursprünglich ausgeschieden haben.

(3) Die das Längenwachstum der Skelettstäbe besorgende Spitzengruppe ändert infolge des Zurückbleibens einzelner Zellkomponenten ihre Zusammensetzung dauernd.

(4) Das Skelettbildnersynzytium von *Echinocyamus* (Gitterstabform) neigt viel stärker als das von *Psammechinus* dazu Plasmafortsätze und Anastomosen zu bilden. Bei der Bildung der analen Gitterstäbe der ersteren Form finden eigenartige rhythmische Plasmabewegungen statt, die jedesmal zur Anlage einer Brücke führen. Dies und anderes erweckt den Eindruck, als wenn das Skelettbildnersynzytium von *Echinocyamus* weniger viskös, also dünnflüssiger ist als das von *Psammechinus*.

(5) Vitalfärbungen zeigen, dass bei Keimblattchimären die Wirts- und Spenderskelettbildner regellos mit einander vermischt werden.

Punkte 1–3 und 5 geben eine vollbefriedigende Erklärung für alle Erscheinungsformen der Keimblattchimären. Insbesondere erklären sich durch den Wechsel der an der Bildung eines bestimmten Skelettabschnittes beteiligten Skelettbildner die von Altrogge (1935) und Schmidt (1936) beschriebenen nachträglichen Veränderungen bereits gebildeter Skelettstücke. Die Mosaikhypothese kann weiterhin nur insoweit in Betracht kommen, als es natürlich oft Abschnitte im Skelett einer Keimblattchimäre gibt, die ausschliesslich von Skelettbildnern des Wirts oder Spenders gebildet sind. Wo aber beide Skelettbildnersortimente vermischt liegen, ist das endgültige Skelettstück das Ergebnis ihres Zusammenarbeitens und somit der Mischwirkung heterozygoter Gene bei den Bastarden durchaus vergleichbar.

Punkt 4 dagegen eröffnet ganz neue Perspektiven, denen wir uns nunmehr zuwenden wollen.

IX. VARIANTEN

Fast allen Forschern, die sich mit Seeigellarven beschäftigt haben, ist es aufgefallen, dass die einfachen Formen (*Paracentrotus*, *Psammechinus*, *Echinus*, etc.) oft Skelettvarianten aufweisen, die insofern gerichtet sind, als sie grossenteils im Auftreten von Skelettstücken bestehen, die den genannten Formen normalerweise fehlen, bei den komplizierteren Gitterstabformen (*Sphärechinus*, *Echinocyamus*, *Echinocardium*, etc.) dagegen vorhanden sind (1931a, 1932a, 1937b). Es handelt sich besonders um das Auftreten oraler Scheitelstäbe und von Parallelstäben in den Analfortsätzen sowie in allerdings seltenen Fällen um die Bildung "unechter" Brücken (Nümann, 1933) zwischen diesen Parallelstäben. Die Varianten haben also gewissermassen intermediären Charakter und ähneln den Bastarden und Keimblattchimären, wie besonders Nümann eingehend beschrieben hat.

Es liegt nahe diese "gerichtete Variation", deren Besonderheit darin besteht, dass sie nicht nur—dem Begriff entsprechend—eine bestimmte Tendenz zeigt, sondern einem Ziel zustrebt, das von anderen Formen bereits erreicht ist, mit den verwandtschaftlichen Beziehungen zwischen den beiden Larventypen in Verbindung zu bringen. Dem steht aber die Tatsache entgegen, dass ein System, das man auf Grund der Ähnlichkeit der Larven aufstellen wurde, keineswegs durchgängig mit dem System übereinstimmt, das man auf Grund des Habitus der Imagines aufzustellen genötigt ist. Es können vielmehr die Larven nahe verwandter Arten wie *Psammechinus* und *Sphärechinus* ganz verschieden, die im System weit von einander entfernter Formen wie *Sphärechinus* und *Echinocyamus* sehr ähnlich sein.

Der oben erwähnte Eindruck, dass das Skelettbildnerplasma der Gitterstabform *Echinocyamus pusillus* weniger viskös sei als das von *Psammechinus miliaris*, legt den Gedanken nahe, dass die Viskosität des Skelettbildnerplasmas einer der wesentlichen Faktoren sei, der die Struktur des Skelettes bestimmt. Diese Annahme fand eine Stütze in der von einer Reihe von Autoren und 1932a ausführlich belegten Tatsache, dass die Skelette einfacher Formen bei erhöhter Temperatur stark zur Ausbildung von Zusatzstäben neigen, die zum Teil den Charakter der oben genannten gerichteten Variationen aufweisen. Es ist aber besonders von Heilbrunn (1927, 1928) nachgewiesen worden, dass eine Erhöhung der Temperatur, innerhalb der angewandten Spanne zu einer Verminderung der Plasmaviskosität von Seeigelkeimen führt. Da nun andererseits bekannt ist, dass man auch auf chemischem Wege eine Viskositätsminderung herbeiführen kann, machte ich den Versuch durch Kombination von Temperaturerhöhung und chemischen Mitteln (Kalilauge—KOH, Natriumjodid—NaJ, Natriumrhodanid—NaSCN) eine verstärkte Viskositätsminderung zu erzielen und zu prüfen, ob die eventuell eintretenden Variationen gerichtet sein würden.

Es ergab sich nicht nur der Arbeitshypothese gemäss eine starke Erhöhung der Skelettvariabilität, sondern dass dieselbe im Wesentlichen in einer Häufung der oben genannten gerichteten Variationen besteht ("Konvergenzskelette").

Abbildung 13 zeigt das rechte und linke Skelett einer normalen *Psammechinus*-Larve, Abbildung 15 das einer *Echinocyamus*-Larve. Abbildung 14 zeigt das

Skelett einer bei einer Temperatur von 18–20 Grad Celsius unter Einwirkung von 0·25 % einer 3 % KalilaugeLösung gezüchteten *Echinus esculentus*-Larve. Wie man sieht, weist die Variante nicht nur anstatt der keulenförmigen analen Scheitelstäbe der einfachen Formen glatte *Echinocyamus*-Scheitelstäbe auf, sondern es sind auch orale Scheitelstäbe vorhanden. Ausserdem sind anstatt der einfachen Analstäbe beiderseits mehrere Parallelstäbe vorhanden, die rechts sogar durch zwei unechte Brücken verbunden sind. Das Ergebnis der Versuche spricht also im Sinne der Arbeitshypothese und deutet auf die Bedeutung der Plasmaviskosität für die Skelettstruktur hin. Eine gewisse Kontrolle des Versuchs lässt sich in der Weise ausführen, dass man Larven einer Gitterstabform derselben Behandlung unterwirft. Handelt es sich um die Folgen einer Viskositätsveränderung, so müsste man auch hier

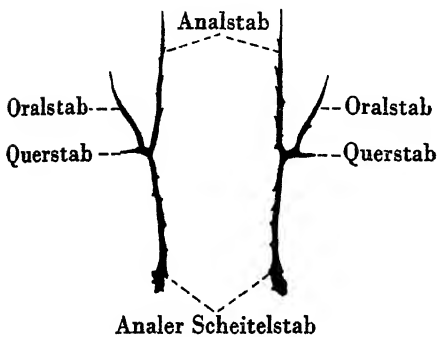


Abbildung 13.

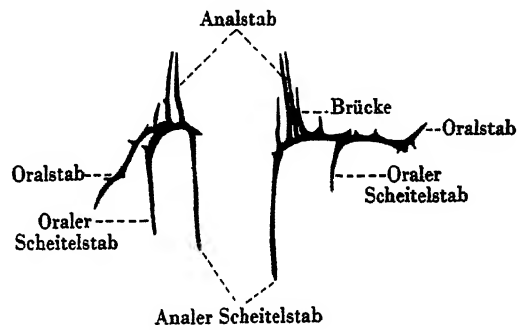


Abbildung 14.

Abbildung 13. Rechte und linke Skeletthälfte einer normalen *Psammechinus microtuberculatus*-Larve.

Abbildung 14. Rechte und linke Skeletthälfte einer *Echinus esculentus*-Larve, die bei 18–20 Grad Celsius unter Einwirkung von 0·25 % einer 3 % KalilaugeLösung gezüchtet wurde. Anale Scheitelstäbe glatt, nicht keulenförmig. Orale Scheitelstäbe vorhanden. Mehrere parallele Analstäbe, rechts 2 unechte Brücken: "Konvergenzskelett".

das Auftreten von Zusatzstäben erwarten. Abbildung 16 zeigt eine derartige Larve von *Echinocyamus pusillus*. Die Abbildung spricht für sich selbst, wenn man sie mit dem normalen Skelett der Abbildung 15 vergleicht.

Das erste Ergebnis dieser Versuche ist eine Erklärung des systematischen Durcheinanders der Larvenformen. Denn es ist natürlich keineswegs gesagt, dass nahe verwandte Formen eine übereinstimmende Plasmaviskosität besitzen müssen. Zweites Ergebnis ist, dass wir einen Schritt weiter in dem Verständnis der Ähnlichkeit der Skelette der Bastarde und Keimblattchimären kommen. Bei den Keimblattchimären mischen sich streckenweise die beiden ordnungsverschiedenen Plasmasyzytien und, eine verschiedene Plasmaviskosität der Ausgangsformen vorausgesetzt, muss ein Gesamtsynzytium mittlerer Viskosität entstehen und folglich ein Skelett intermediären Aussehens. Bei den Bastarden ist nur das mütterliche Plasma mit seiner speziellen Plasmaviskosität vorhanden. Entstehen trotzdem Skelette intermediären Charakters, so kann das nur darauf beruhen, dass in den Kernen die Anlagen der beiden ordnungsverschiedenen Eltern vereinigt sind. Die heterozygoten Kerne müssen ihrerseits die Plasmaviskosität in intermediärem Sinne

beeinflusst haben. Dies zeigt, dass letzten Endes die Plasmaviskosität normalerweise genkontrolliert ist (1937b). Aber wir können die Plasmaviskosität auch durch äussere Einflüsse verändern. So erklärt sich hier die auch in so vielen andern Fällen beobachtete phänotypische Übereinstimmung von Varianten und Mutationen.

Selbstverständlich kann der Viskositätsunterschied nicht allein für alle Verschiedenheiten des Gesamtskeletts der Seeigellarven verantwortlich gemacht werden. Es müssen weitere Erbfaktoren, die Besonderheiten des Skeletts, Lage und Beschaffenheit der ektodermalen Attraktionszonen und die Empfindlichkeit der Skelettbildner gegenüber den Einflüssen der Attraktionszonen betreffen, vorhanden sein (1937b).

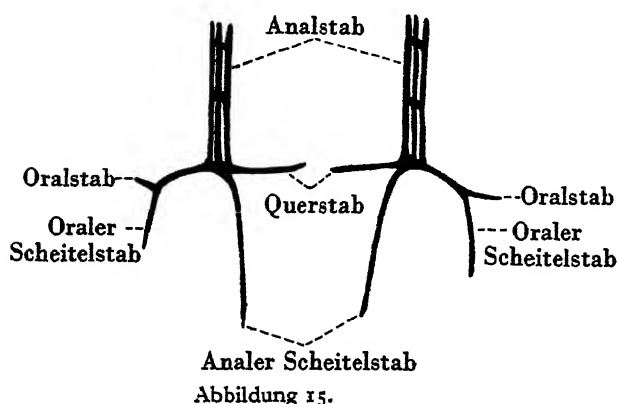


Abbildung 15.

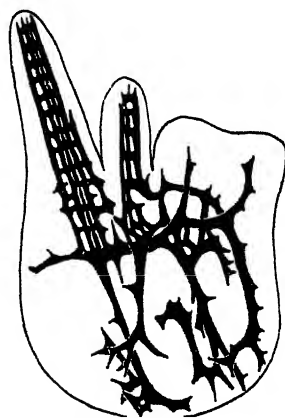


Abbildung 16.

Abbildung 15. Rechte und linke Skeletthälfte einer normalen *Echinocyamus pusillus*-Larve.

Abbildung 16. *Echinocyamus pusillus*-Larve, die bei 25 Grad Celsius unter Einwirkung von 0.25 % einer 3 % Kalilauge-Lösung gezüchtet wurde. Skelett überreich entwickelt.

Bereits 1932a habe ich die Skelette verschiedener Seeigellarven nach ihrer Kompliziertheit in einer aufsteigenden Reihe zusammengestellt und diese mit den von andern Forschern an anderen Objekten nachgewiesenen orthogenetischen Entwicklungsreihen verglichen. Es musste damals die Einschränkung gemacht werden, dass solche bei andern Formen "realiter erhalten" worden sind, "während wir sie bei den Pluteis nur erschliessen" (S. 248). Die Viskositätsversuche zeigen, dass wir sie nunmehr auch bei den Pluteis als Modifikationen realiter herstellen können.

Die hier dargelegte Viskositätstheorie ist weiterer Prüfung auf verschiedenen Wegen zugänglich.

X. ZUSAMMENFASSUNG

Wenn man die Skelettbildner eines Seeigelkeims (Spender) in den Keim einer andern Art (Wirt) transplantiert, dessen Entoderm und Mesoderm vorher entfernt ist und der also nur Ektoderm enthält (Fragmentkeim), so erhält man eine "reine

Keimblattchimäre", die nur aus Haut und Skelett besteht. Das Spenderskelett ist in der Wirtslarve harmonisch gelagert, richtet sich also in dieser Hinsicht nach dem Wirt. Andererseits wirkt es auf denselben insofern ein, als es den Wirt zur Bildung von Larvenfortsätzen veranlasst. Die Struktur des Skelettes entspricht der Spenderform.

Werden die Skelettbildner in eine Ganzlarve einer andern Form implantiert ("Keimblattchimären"), so entsteht ein intermediäres Skelett mit Ausnahme der Skelettstäbe, die nur bei der Wirtsform vorkommen. Diese werden in reiner Wirtsform ausgebildet.

Implantiert man die Skelettbildner in einen Keim, dem man vorher die eigenen Skelettbildner fortgenommen hat, so entwickelt sich zunächst ein Skelett, das die Struktur des Spenders aufweist. Nachträglich liefert auch der Wirt regulativ Skelettbildner und das bereits gebildete Skelett geht mehr und mehr in die Wirtsform über ("Stockwerkchimären").

Stellt man durch Kreuzbefruchtung Bastarde aus denselben Formen her, aus denen die Chimären zusammengesetzt sind, so weisen diese ebenfalls intermediäre Skelette auf.

Stellt man sich einen Bastard her ($A \text{ ♀} \times B \text{ ♂}$), so enthalten dessen Skelettbildner nur mütterliches (A) Plasma, aber zur Hälfte mütterliches (A) und zur Hälfte väterliches (B) Chromatin. Implantiert man solche Bastardskelettbildner in einen Keim der mütterlichen Art (A), deren Skelettbildner nur A-Plasma und A-Chromatin enthalten, so erhält man eine "Bastardchimäre". Die Skelettbildner der Bastardchimäre enthalten nur Plasma der mütterlichen Art (A), aber Chromatin beider Arten im Verhältnis 3A : 1B. Die Skelette sind intermediär, nähern sich jedoch in ihrer Struktur mehr der mütterlichen Art. Schwächt man die mütterliche Komponente, indem man dem Wirt vor der Implantation einen oder mehrere Skelettbildner wegschneidet, so wird die Intermediarität des Skeletts entsprechend stärker ausgeprägt.

Die Bildung eines Skelettstabes ist abhängig von zwei Faktorenpaaren: Dem Vorhandensein des Fortsatzektoderms und der betreffenden Skelettbildner. Fehlt das Fortsatzektoderm, so kann der betreffende Skelettstab nicht gebildet werden. Ist das Fortsatzektoderm vorhanden, die Skelettbildner aber Bastarde von einer Art, die den betreffenden Stab normalerweise besitzt, und einer solchen, die ihn nicht besitzt, so wird der Stab nicht gebildet. Das Fehlen des Faktors Skelettbildner ist also dominant über sein Vorhandensein.

Die Analyse der normalen Skelettbildung erweckt den Eindruck, dass das Skelettbildnerplasma von *Echinocyamus* eine geringere Viskosität besitzt als das von *Psammechinus*. Die Plasmaviskosität scheint einer der Faktoren zu sein, der die verschiedene Struktur der Skelette bestimmt.

Hierdurch erklärt sich, dass unter den Seeigellarven der einfachen Arten "gerichtete Varianten" auftreten, die dem Typus der komplizierteren Formen zustreben. Durch Einwirkung erhöhter Temperatur und chemische Beeinflussung gelang es Larven von *Echinus* weitgehend in der Richtung auf *Echinocyamus* abzuändern ("Konvergenzskelette").

Die Intermediarität der Bastarde beruht darauf, dass in den Kernen der Skelettbildner die Anlagen der beiden Elternarten vereinigt sind. Die Kerne kontrollieren ihrerseits die Viskosität des Skelettbildner-Plasmasynzytiums, in dem sie liegen.

Die Intermediarität der Chimären beruht darauf, dass Kerne beider Ausgangsarten in einem Mischplasmasynzytium beider Arten liegen, das folglich mittlerer Viskosität ist.

Die Intermediarität der Varianten beruht darauf, dass durch äussere Einflüsse die Viskosität des Skelettbildnerplasmas verändert wird.

XI. SUMMARY

When skeleton-forming cells of a donor sea urchin embryo are transplanted into a host embryo of another species, whose endoderm and mesoderm have previously been removed so that it only possesses ectoderm, a larva may be produced which is a chimaera consisting only of skin and skeleton. The donor skeleton is harmoniously situated in the host larva, thanks to the influence of the latter. But the skeleton affects the host inasmuch as it forces the latter to form larval processes. The skeletal structure is of the donor type.

When skeleton-forming cells are implanted into the whole larva of another species, an intermediate type of skeleton arises, with the exception of the skeletal rods, which occur only in the host form. These are developed exactly as in the host form.

When skeleton-forming cells are implanted into an embryo whose own skeleton-forming cells have previously been removed, a skeleton develops which at first has the donor structure. Later on the host also supplies skeleton-forming cells, and the skeleton which has already been formed gradually changes towards that of the host form.

Hybrids obtained by cross-fertilization of the same forms as those which made the chimaeras also have intermediate skeletons.

When a species-hybrid is made ($A \varnothing \times B \sigma$), its skeleton-forming cells contain only maternal cytoplasm (A), but half maternal (A) and half paternal (B) chromatin. When the skeleton-forming cells of such a hybrid are implanted into an embryo of the maternal species (A), whose skeleton-forming cells contain both A cytoplasm and A chromatin alone, a hybrid chimaera is obtained, the skeleton-forming cells of which contain cytoplasm of the maternal species (A) alone but chromatin of both species in the ratio of $3 A : 1 B$. The skeletons are intermediate, but approach nearer to the maternal type. If the maternal component is weakened by the excision of some skeleton-forming cells from the host before the implantation, then the skeleton is more definitely intermediate.

The formation of a skeletal rod depends on two factors, the presence of the arm-ectoderm and of the corresponding skeleton-forming cells. If the arm-ectoderm is absent, the corresponding skeletal rod cannot be formed. If the arm-ectoderm is present, and the skeleton-forming cells are hybrids between a species which normally possesses a skeletal rod and one which lacks it, then the rod is not formed. Thus the absence of the skeleton-forming factor is dominant to its presence.

A study of normal skeleton formation gives the impression that the skeletogenous cytoplasm of *Echinocyamus* has a lower viscosity than that of *Psammechinus*. Protoplasmic viscosity seems to be one of the factors determining the particular structure of the skeleton.

The fact that in sea-urchin larvae with simple skeletons there appear "directed variations" tending towards the type of the more complicated forms is explicable in this manner. It was found to be possible, through the effects of high temperature and chemical substances, to influence larvae of *Echinus* very considerably in the direction of *Echinocyamus*.

The intermediate nature of hybrids is due to the fact that the nuclei of the skeleton-forming cells contain elements of both parental species. These nuclei control the viscosity of the skeletogenous syncytium in which they lie.

The intermediate nature of chimaeras is due to the nuclei of both species being placed in a mixed syncytium belonging to both species, which consequently has an intermediate viscosity.

The intermediate nature of variants is due to the viscosity of the skeletogenous protoplasm having been altered by external means.

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ERRATUM

BIOLOGICAL REVIEWS, Vol. XIII, p. 412

Table IV

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A SURVEY OF INTERACTION BETWEEN FUNGI

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(Received 25 May 1938)

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I. INTRODUCTION

ONLY a few problems concerning interaction between fungi have received intensive study, and these, as a rule, because the results seemed likely to have economic importance in the near future. Frequently, however, interaction between fungi has been found to provide the basis for explaining certain aspects of phenomena that were otherwise obscure, and has received incidental treatment for this reason. Accordingly, the literature on interaction is dispersed and fragmentary, and, although the subject is still in an early stage of development, it has seemed important to survey the scattered results as far as possible.

The need for a uniform terminology is stressed by the confusion already existing. The word "interaction" has a meaning wide enough to include all the possible effects peculiar to mixtures of micro-organisms. "Antagonism" has been used

frequently in this connotation, but could be reserved with advantage for the type of interaction in which one partner in the mixture exerts an adverse influence on the other; in contrast to this type, the term "association" should be applied when there is growth together without any harmful mutual influence.

Unger (1833, p. 173) was impressed by an apparent connexion between the occurrence of two plant parasites, perhaps the earliest account of an example of interaction between fungi. He observed that *Botrytis pygmaea* (later changed to *Plasmopara pygmaea*) only appeared on the lower surface of leaves of *Anemone ranunculoides* when the same surface had been attacked by *Aecidium punctatum*. In 1863 Pasteur demonstrated the coordinated association of aerobic and anaerobic organisms in nature, the first example of microbial association to be elucidated. This paper will deal principally with interactions between fungi, since the field of bacterial interaction has been reviewed by several authors. Nicol (1934) gave a brief general treatment of the growth together of bacteria under natural and semi-natural conditions and under culture in the laboratory. The effects of bacterial interaction in culture media have been summarized by Buchanan & Fulmer (1930). Holman in Jordan & Falk's book (1929) has reviewed some types of interaction in medical bacteriology.

The work of Winogradsky on the bacterial soil flora, especially in its relationship to nitrification (1891, 1933) and to the decomposition of organic matter (1924), led to a growing realization of the importance of interaction under natural conditions. Russell (1932) and others have also stressed the importance of antagonism between soil micro-organisms. Recently, Fawcett (1931) asserted that "nature does not work with pure cultures alone, but most frequently with associations". He emphasized the importance of two phases of research, namely "the study in a quantitative as well as a qualitative way of the effect of known mixtures of micro-organisms in culture media;" and, secondly, "the study in the same way of the effect on the development of disease by inoculation of plants with known mixtures of micro-organisms". He reviewed the literature briefly under these two headings, which represent two technical approaches to the study of interactions. The nature of the substrate in which interaction occurs would seem to provide a more natural criterion for subdivision of the subject. On this basis three principal types of interaction may be distinguished as occurring (1) in the soil, (2) on or in a host plant, and (3) on artificial media. Each of these three subdivisions will be considered in turn.

II. INTERACTION IN THE SOIL

(1) INTERRELATIONSHIPS WITHIN THE GENERAL SOIL MICROFLORA

Waksman (1937), in an extensive historical review, has dealt particularly with the antagonistic reactions of bacteria with one another, and of bacteria with fungi. He stated that the extent of development of any one group of micro-organisms inhabiting a natural substrate depends on (a) the food supply, both qualitative and quantitative, (b) the environmental conditions, and (c) the presence and abundance

of other organisms capable of producing stimulating or toxic substances, or of parasitizing the organism in question.

Rege (1927) has emphasized the importance of fungi in the decomposition of cellulosic material. He found that, under the conditions of his experiments, and in the presence of available nitrogen, certain fungi together produced a decomposition at least as rapid and complete as that effected by a normal soil microflora, while bacteria alone were very considerably slower. Norman (1930) studied the availability of various nitrogen and carbon compounds to certain cellulose-decomposing fungi, and showed that simple mixtures of these fungi might be either competitive or cooperative in thermogenic power, depending primarily on the interrelationships of their nutrient requirements. Waksman & Hutchings (1937) studied the associative growth of different fungi, actinomycetes and bacteria upon various plant materials. Certain organisms showed clear characteristics; for instance, actinomycetes could not attack corn stalks, but were the only organisms capable of decomposing lignin. The growth of one organism was, however, considerably modified in the presence of other organisms; and many stages of decomposition were performed more rapidly by mixtures of micro-organisms, although only one of the organisms was able to perform the principal reaction.

In the vicinity of living plant roots the number of soil micro-organisms becomes rapidly greater the nearer the sample is taken to the root, and reaches a maximum on scrapings taken from the surface of the roots (Thom & Humfeld, 1932). Melin (1925) showed that excretion of phosphatides by roots could cause local stimulation of fungus growth in the root zone, and suggested that this might account in part for the phenomena of ectophytic mycorrhiza. Recently, Burges (1936) has emphasized the view that the mycorrhizal fungus is a weak pathogen, and states that the association "is to be regarded as an example of controlled parasitic attack and has no mutualistic significance". Wherefore, in common with other diseases caused by soil fungi, the general soil microflora would be of prime importance in the interpretation of mycorrhiza. Nevertheless, as Waksman (1937) concludes, "the available information concerning antagonism among micro-organisms may be useful in explaining the behaviour of various specific organisms; it is little more than suggestive, however, in explaining the mutualistic interrelationships of the numerous micro-organisms comprising the soil population and the equilibrium normally found to exist in this population."

(2) INTERACTIONS INVOLVING SOIL-BORNE PATHOGENS

The influence of the general soil microflora on specific organisms is of tremendous importance in studying diseases caused by certain soil-borne pathogens. The interaction of soil micro-organisms not only provides an interpretation of the underlying principles of certain long established agricultural practices, but also gives a clue to their extension for the prevention of crop diseases. This is illustrated by the control of common scab of potatoes by green-manuring.

Millard (1923) showed that green-manuring could be very beneficial in controlling potato scab, and suggested that the causal organism, *Actinomyces scabies*,

was only parasitic in the absence of suitable organic nutrient in the soil to sustain it as a saprophyte. Subsequently Millard & Taylor (1927) found this "preferential food hypothesis" untenable, since green-manuring exerted no inhibitory effect on scab in the absence of purely saprophytic *Actinomyces* species; inoculation of the soil with the saprophytic species *A. praecox* resulted in inhibition of scab, which became successively more complete as the inoculum of *A. praecox* was increased in amount. Evidently *A. scabies* was eliminated from the soil when there was sufficient organic nutrient to stimulate the competing saprophytic species. It was suggested that bacteria also play a part in suppressing *A. scabies* under field conditions. Sanford (1926) found that spores of *A. scabies* germinated best at pH 8.5, and would not germinate at a more acid reaction than pH 5.3; he showed that pure strains of bacteria cultured in the same solution with *A. scabies* produced a reaction too acid for the germination of the latter. Certain bacteria produced greater acidity when cultured with *A. scabies* than in pure culture; and one bacterial strain inhibited the development of *A. scabies* by some means other than excessive acidity. Hence, the elimination of the pathogenic *A. scabies* may be due in part to the production of too acid a soil reaction by the predominating soil saprophytes, which become abundant in the presence of organic nutrients.

In the last decade foot and root rots of wheat have been the subject of much investigation. Surveys in several countries have agreed in finding *Ophiobolus graminis* the most frequent pathogen, although a similar disease is sometimes caused by *Helminthosporium sativum*, and, less frequently, by several other pathogenic fungi. Attempts to inoculate wheat plants with *Ophiobolus graminis* under field conditions met with no success; it appeared that the fungus was quickly rendered inoperative when incorporated in the soil. Sanford & Broadfoot (1931) found that many soil-inhabiting fungi and bacteria were able to suppress the pathogenicity of *O. graminis* in pot culture at 20° C. Filtrates from cultures of some of the fungi and bacteria also possessed the power of suppressing the pathogenicity of this fungus, though to a less degree. It was, therefore, presumed that these organisms produced during their growth substances toxic to *O. graminis*. Henry (1931), by comparing the results of inoculations in sterilized and unsterilized soil, showed that the natural microflora of Edmonton, Alberta, strongly inhibited *Helminthosporium sativum*, and also *Fusarium graminearum*, another pathogen causing foot rot of wheat. Fungi isolated from this soil (the fungi were estimated at only 1-3% of the total count by Bisby *et al.* 1933) were considerably more inhibitory than bacteria or actinomycetes; a combined inoculum of all three was as effective as unsterilized soil.

Temperature has an important influence on the interaction between pathogen and soil microflora (Henry, 1932). At 13° C. the incidence of *Ophiobolus graminis* was about equally severe in sterilized and unsterilized soil; at 27° C. most of the seedlings in sterilized soil were killed, while those in unsterilized soil were little harmed. Garrett (1934a, 1936) has shown that *O. graminis* is very susceptible to changes in the soil environment, although temperature probably affects the pathogen principally through its action on the general soil microflora. He also emphasized the influence of the kind of inoculum, type of soil, and temperature, moisture

content and degree of sterilization of the soil, on the antagonism between the pathogen and other saprophytes (Garrett, 1934b).

Brömmelhues (1935) investigated the interaction on solid and liquid culture media between *O. graminis* and a number of other soil-inhabiting fungi. The growth of *O. graminis* proved to be effectively inhibited by *Helminthosporium sativum* and *Penicillium* species in particular. In pot experiments the most severe damage was caused by *Ophiobolus graminis* on plants which had been inoculated four weeks previously with most of the fungi used in the tests on artificial media; *Cladosporium herbarum*, especially, secreted virulent toxins which lessened the resistance of the host. On the other hand, plants grown in pots inoculated with *Ophiobolus graminis* and *Helminthosporium sativum* or *Penicillium* species together suffered less than control plants inoculated with *Ophiobolus graminis* alone; but if weakly antagonistic forms were inoculated at the same time as *O. graminis*, the damage caused by the latter was increased. Brömmelhues attributed these results to the action of the toxins produced by the soil saprophytes on both the host roots and the pathogen. On the basis of his experimental data, Garrett (1936) proposed a different hypothesis, namely that the growth of *O. graminis* along the roots is depressed by any increase in the carbon dioxide concentration in the microclimate of the root zone. Carbon dioxide accumulates from the respiration of the roots of the host plant, of the hyphae of *O. graminis* and of the soil microflora. According to this hypothesis the respiration of soil saprophytes is of prime importance, rather than their production of toxins.

Garrett (1936) has emphasized that *O. graminis* appears to be limited in its saprophytic powers to growth on dead tissues which it has killed. Padwick (1935) found that it could spread little, if at all, in soil bare of vegetation. *O. graminis* was isolated from infested soils to a depth of 10 in. by Fellows (1929), and, subsequently, to a depth of 15 in. by Fellows & Ficke (1934). The latter authors sowed wheat seed in non-infested soil inoculated at different levels with *O. graminis*, and found that the wheat plants only became infested when the fungus had been placed within three inches of the seed. Napper (1934) found that the rubber root-rot fungi, *Fomes lignosus*, *F. noxius* and *Ganoderma pseudoferreum*, could not grow through the soil except along an extended object, preferably a root surface. Again, Sanford (1933a) in his studies on potato scab showed that the spread of *Actinomyces scabies* through the soil is negligible. On the other hand, an initial infection of *Phymatotrichum omnivorum* can spread 4.5 m. radially in 50 days (King, 1923); this rapid spread presumably occurs mostly along the roots of the cotton plants.

(3) INTERACTION AS A CONTROLLING FACTOR IN SOIL-BORNE DISEASES

As yet there is no evidence to show whether suppression of the pathogenicity of *O. graminis* in the soil is due to decrease in quantity of this pathogen in the soil, or whether its virulence and not its abundance is reduced (Sanford, 1933b). An understanding of the conditions favourable to epidemics of foot and root rots must rest on the elucidation of the effect of external factors on the equilibrium constitution of the soil microflora, and of the effect of changes in the soil microflora on its

interaction as a whole with the pathogen under consideration. The amount and kind of food, the reaction, temperature and moisture content of the soil, and other such factors, are known to influence the soil microflora profoundly (Waksman, 1932). In consequence, diseases caused by soil pathogens can be controlled to some extent by cultural practices. Recently, Fellows (1929) demonstrated that the addition of different kinds of organic matter (e.g. chicken or horse manure, alfalfa stems and leaves, etc.) to the soil greatly reduced the severity of take-all, giving almost perfect control in some cases. King *et al.* (1934) showed that green alfalfa manure was strikingly effective in controlling cotton root-rot under irrigation conditions in Arizona. Carbon dioxide was evolved considerably more rapidly from the manured plots than from the unmanured controls, and this increased respiration was attributed to the increase of the soil microflora in the presence of organic manure. In addition, a direct study of the soil microflora by the Cholodny slide technique showed that bacteria, actinomycetes and most fungi were more abundant in the manured plots, while *Phymatotrichum omnivorum*, the causal organism of cotton root rot, was more abundant in the unmanured plots. There is evidence from many countries (Garrett, 1936; Brömmelhues, 1935; Moritz, 1932) that take-all is worst on the lighter soils, and on alkaline soils; the disease is also favoured by manurial deficiencies, and by any treatment tending to open up the soil. The authors mentioned above discuss possible applications of these data to control systems based on manuring, soil treatment and crop rotation.

As early as 1918 it was demonstrated (Hartley *et al.* 1918; also Hartley, 1921) that saprophytic soil fungi might play a role in decreasing the damping-off of conifer seedlings. In 1932, during an investigation of the damping-off disease of citrus seedlings caused by *Rhizoctonia solani*, Weindling (1932) discovered that this and other pathogenic soil fungi were parasitized by a strain of *Trichoderma lignorum*. From a study on nutrient media he showed (Weindling, 1934a) that the interaction between *T. lignorum* and various soil fungi was dynamic, and could be transformed by changes in external conditions from compatibility to antagonism or even parasitism. The antagonism was attributed to a lethal principle, which was produced by most strains only at highly acid reactions (Weindling, 1934b). A number of other soil fungi proved capable of a similar type of parasitic action on *Rhizoctonia solani* by means of toxic substances. Pot experiments suggested the possibility of biological control of seed decay and damping-off by heavy inoculation of the soil with strains of *Trichoderma lignorum* (Bisby *et al.* 1933; Weindling & Fawcett, 1934; Allen & Haenseler, 1935). Weindling & Fawcett (1936) obtained successful control of the damping-off of citrus seedlings by *Rhizoctonia solani* under laboratory, greenhouse and field conditions by applying aluminium sulphate to the soil layers adjacent to the seeds. This treatment provided an initial soil reaction of about pH 4.0, which favoured the effectiveness of such organisms as *Trichoderma* in the soil microflora, and so increased antagonism against *Rhizoctonia solani*; at neutral reactions there was no biological control of this disease. Within the range of 18 to 35°C., temperature affected neither the severity of damping-off due to *R. solani*, nor the degree of control of the disease resulting from the above treatment.

The importance of the soil microflora in modifying diseases caused by a number of other soil-borne pathogens is now coming to be realized. Brown (1933) found a reduction of the incidence of the watermelon disease caused by *Phymatotrichum omnivorum* when certain fungi and bacteria were present in the soil with the pathogen; and, in culture, *Trichoderma lignorum* was observed to attack and kill the hyphae of *Phymatotrichum omnivorum*. The severity of the seedling blight of flax, due to *Fusarium lini*, was diminished when the pathogen was accompanied in the soil by certain other fungi (Tervet, 1938). Greaney & Machacek (1935) demonstrated by a series of pot experiments that the pathogenicity of *Helminthosporium sativum* on wheat seedlings was suppressed by the antagonistic action of *Cephalothecium roseum*; preliminary physiological studies suggested that this suppression was due to toxic staling substances produced by *C. roseum*. Tims (1932) found that a number of actinomycetes were antagonistic to a *Pythium* root parasite of sugar cane, when inoculated into sterile soil in greenhouse tests; the antagonism was independent of pH changes, and was attributed to a toxic principle, which was moderately thermostable. Van Luijk (1934) showed a marked influence of the soil microflora on grass diseases caused by species of *Pythium*. Endo (1931, 1932a, b, 1933, 1935) listed numerous soil micro-organisms which were moderately or strongly antagonistic to *Hypochnus centrifugus*, *H. sasakii* and *Sclerotium oryzae-sativae* in culture media and in the soil; and pot experiments showed a reduction in the pathogenicity of these fungi. It was further demonstrated that culture filtrates from certain of the antagonistic fungi were also able to lessen the damage caused by the three pathogens mentioned above. A number of cases of antagonism have been reviewed by Hino (1935), who has indicated the possibilities of biological control of certain soil-borne diseases in Japan. The existing control methods for soil-borne fungus diseases of field and plantation crops have been reviewed by Garrett (1937).

III. INTERACTION ON OR IN A HOST PLANT

Interactions between organisms within or on the surface of host tissue differ from interactions occurring in the soil in three important respects: firstly, interaction usually occurs only when the organisms are closely adjacent; secondly, interactions of this type are generally restricted to a small number of organisms, often only two, under natural conditions; and, thirdly, the substrate is relatively constant and homogeneous, if complex, in composition. Moreover, the interaction may be more or less limited in duration, and sometimes differs in type at different phases of the life-cycle of one of the fungi. On the basis of the duration and intensity of interaction, examples could be placed in a series ranging from direct parasitism, through successional disease, to continued growth in association.

(1) PARASITISM AND SUCCESSIONAL DISEASE

There are numerous recorded instances of parasitism of one fungus by another. Koch (1934a) observed that the conidial-bearing stroma of *Dibotryon morbosum* on many host plants was parasitized every summer by *Cephalothecium roseum*, which

exerted a measure of biological control. Ten other fungi were frequently isolated from the stromata; one of these, a species of *Coniothyrium*, regularly produced numerous pycnidia around and within the perithecia of *Dibotryon morbosum*, yet did not prevent the development of the latter. The parasitism of one fungus by another is too large a subject to be considered here; a number of instances are described by Blochwitz (1930); beyond these it will suffice to mention two examples which suggest eventual possibilities of control of some diseases by this means. Cereal rusts are known to be attacked by a number of micro-organisms under field, and, especially, under greenhouse conditions (Levine *et al.* 1936). *Cronartium ribicola* on *Pinus strobus* in Scotland is sometimes attacked by another fungus, *Tuberculina maxima*, which prevents the rust from sporulating (Brooks, 1928, p. 252).

Bamberg (1931) described ten cultures of bacteria capable of inhibiting the development of *Ustilago zeae* on artificial media. One of these was investigated further and shown to prevent the production of smut galls, if inoculated into corn plants at the same time as sporidia of *U. zeae* or within three days earlier or later. Filtrates of the bacterial cultures did not prevent smut development. Johnson (1931) investigated the physiology of four of the antibiotic bacteria, and found that some of them contained enzymes capable of dissolving the chemical constituents of the sporidial cell walls. However, other bacteria with the same enzymes did not affect the sporidial cell walls; hence, the antibiotic properties of the inhibiting bacteria could not be attributed to their enzyme complements.

Successional diseases may occur on the living host through the action of one fungus in developing a substrate which is more favourable for a second fungus, a process comparable to the successive metabolic stages of decay under natural conditions. Frequently, one parasite by its attacks on a plant allows the entrance of a more virulent parasite, which could not by itself have initiated the attack. For example, Alcock (1926) described a disease of young shoots of *Salix fragilis* caused by the fungus, *Cryptomyces maximus*. Another pathogen, *Scleroderris fuliginosa*, could only gain entrance through the lesions caused by *Cryptomyces maximus* and several other fungi, but subsequently was able to spread destructively. On the shoots attacked by these two fungi, the areas of injury were increased in a semi-saprophytic manner by a third fungus, *Myxosporium scutellatum*. Brooks (1935, p. 134) has reported that the first fungus on a newly exposed oak stump is usually *Stereum hirsutum*, and on birch stumps the first fungus is commonly *S. purpureum*. Moreover, the permeation of oak wood by *S. hirsutum* appeared to prevent the subsequent development of *S. purpureum*, just as infection of exposed plum wood by comparatively harmless micro-organisms made conditions unfavourable for the virulent pathogen, *S. purpureum*.

(2) PROLONGED INTERACTION BETWEEN TWO ORGANISMS

In contrast to these interactions of short duration resulting in the establishment of one fungus, there are numerous instances of prolonged interaction, some occurring between bacteria, others between fungi, and yet others between bacterium and fungus. As far as is known, bacteria and fungi, as a whole, do not have funda-

mentally different types of metabolism, and the nature of an interaction would seem to depend principally on the physiologic characteristics of the particular organisms involved, and on the medium in which the interaction takes place.

Burkholder & Guterman (1932) described in full detail two distinct types of bacteria isolated from single lesions on *Hedera helix*. Only one of these, named *Phytomonas hederæ*, was pathogenic when inoculated alone; the other acted as a temporary accelerator in combination with the pathogen, the degree of acceleration increasing with a rise in temperature. Two bacteria amongst other micro-organisms were isolated from sugar canes afflicted with a disease causing sudden wilting (Desai, 1935); one gave bluish and the other white colonies on nutrient agar. Inoculations proved the former to be pathogenic and the latter inactive; mixed cultures were, however, much more virulent than the pathogenic bacterium alone. Dowson & d'Oliveira (1935) reported the occurrence in England of *Aplanobacter rathayi* on *Dactylis glomerata*. They found that the naturally occurring slime was pathogenic, but not pure cultures of the organism, and observed the presence of smaller numbers of two other types of bacteria in the isolation plates. In each of these instances the virulence of the pathogen was increased by association with other bacteria. In contrast, Adam & Pugsley (1935), in their investigations on the halo blight disease of French beans in Victoria, reported that a yellow bacterium sometimes became associated systemically with the pathogen, and retarded and mitigated the severity of the disease symptoms. A bacterium with characters similar to this yellow bacterium was isolated from lesions on other plants, and exerted the same adverse effect on the halo blight pathogen.

Fawcett & Lee (1926) inoculated branches of the walnut with mixtures of the bacterium *Pseudomonas juglandis*, which is responsible for a blight of the leaves and young stems, and the fungus *Dothiorella gregaria*; the pathogenicity of the fungus was inhibited by the bacterium.

Nicolas & Agg ry (1933) described the association of two kinds of bacteria with the pathogen *Phyllosticta daphniphylli* in lesions on *Daphniphyllum glaucescens*. The symptoms seemed to result from both fungus and bacteria, but the role of each was not analysed.

Davidson (1928) made the interesting observation that stocks of the potato variety Champion, which have remained free from Mosaic virus, are still markedly resistant to attack by *Phytophthora infestans*, as in the early days of the cultivation of this variety in Ireland.

Many examples of associative growth concern fungi causing rots of soft fruits. Stevens (1916) observed that two fungi frequently infected the same strawberry. Certain fungi mingled freely, as *Fusarium* with *Botrytis* or *Rhizopus*; other fungi were separated by a conspicuous inhibition zone, as *Botrytis* with *Alternaria*. Machacek (1928) studied the interactions between pairs of fungi, of which two had been isolated from apples, two from tomatoes and three from carrots. The types of interaction on artificial media depended largely on temperature and on the relative amounts of inoculum of each fungus, and were often widely different from the interactions of the same mixture of fungi growing in host tissue. Those fungi which

inhibited the growth of another fungus appeared to do so by the secretion of a toxic substance, which was not an acid, and which was partially destroyed by autoclaving.

Gioelli (1932) reported that oranges, lemons and mandarins in Italy were frequently attacked by both *Penicillium digitatum* and *P. italicum*; the two fungi were antagonistic, but *P. digitatum* grew more rapidly and surrounded the area infected by *P. italicum*. In Palestine, Reichert & Hellinger (1932) observed that infection of overripe citrus fruits by *Diplodia natalensis* was considerably increased in the presence of *Penicillium digitatum* and *P. italicum*. Experiments showed that a mixture of either or both of these species with the inoculum of *Diplodia natalensis* caused more rotting than did the latter fungus by itself.

Fawcett (1913, 1923) described several instances in which inoculations with a mixture of two citrus fungi caused more severe injury than inoculation with either fungus alone. Moreover, lesions on citrus trees caused by *Phytophthora citrophthora* in combination with a *Fusarium* species enlarged more rapidly than those caused by *Phytophthora citrophthora* alone, although the *Fusarium* by itself was non-pathogenic.

Savastano & Fawcett (1929) observed that the symptomatology of naturally occurring citrus decay often differed from pure culture infections made in the laboratory. Using twelve cultures of fungi parasitic on citrus fruits, they compared the amount of rotting after a given time caused by inoculations with mixed spore suspensions of two fungi (and, occasionally, of three fungi) with the amounts of rotting caused by each fungus separately; each experiment was performed at seven constant temperatures, covering approximately the range from 10 to 30° C. With certain combinations of fungi the rate of decay was markedly greater than that caused by the faster-growing organism alone; other combinations produced a rate of decay approximating to that of the faster-growing component by itself; with yet other mixtures the rate of decay was less than that resulting from the slower-growing component by itself. With most mixtures temperature had an important influence on the type of interaction. The authors considered that "the mechanism of this depression or acceleration of the mixture is probably related to the combinations of enzymes that are present and their action in making food material for growth available or in producing inhibiting substances. The specific food requirements of the respective fungi and the competition which these imply are probably of prime importance in attempting an explanation of the behaviour of the fungi in mixtures."

Vasudeva (1930) inoculated apples with a parasitic fungus, a non-parasitic fungus, and a mixture of the two; the results of nine such experiments with different pairs of fungi gave in each case a significant lowering of the amount of attack of the parasitic fungus in the presence of the saprophyte, due to the interference of one fungus with the growth of the other. However, an analysis of the interaction between *Monilia fructigena* and *Botrytis allii* showed that the activity of the former could be reduced by culture filtrates of the latter. Metabolic products of *B. allii* retarded greatly the germination of conidia of *Monilia fructigena*, and this might account in part for the phenomenon.

Both Vasudeva and Savastano & Fawcett studied the effect of interaction by comparing the amount of rotting caused by the mixture with that caused by each

fungus separately after a certain length of time. In order to elucidate the relations between two apple-rotting fungi at different stages in the progress of the mixed rot, the writer used an accurate method to determine the amounts of rotting in statistically comparable samples at intervals after inoculation (D'Aeth, 1937). Comparisons of the rates of development of rots caused by a mixture of *Sclerotinia fructigena* and *Penicillium expansum* and by each of these fungi separately showed that interaction occurred principally during the early phase of development of the rot; the type of interaction appeared to vary with changes in temperature and degree of ripeness of the apples. During the late phase of rotting the two fungi in the mixture appeared to grow independently.

There is some evidence that fungus spores have an important influence on the germination of one another. Doran (1919) observed reduction of germination of certain rust spores in the presence of spores of other fungi. He attributed the failure of teleutospores of *Puccinia malvacearum* to germinate in the presence of spores of several saprophytes (Doran, 1922) to interference with the oxygen supply. Satoh (1931) found that the culture liquid of *Ophiobolus miyabeanus* contains two kinds of substances, one accelerating and the other retarding the germination and growth of *Aspergillus niger*. The former is thermostable, and will pass through a Chamberland filter. Both substances produce their maximum effects during the first week of growth of *A. niger*.

(3) HOST REACTIONS TO PATHOGENS

There is one complicating factor which deserves some consideration—the reaction of the host to the pathogens. Several workers (Johnston, 1934; Mains, 1934; Roberts, 1936) have observed that large pustules of leaf rust, *Puccinia triticina*, may develop in the immediate vicinity of patches of mildew, *Erysiphe graminis*, on leaves of a wheat variety which is normally highly resistant to the particular physiologic form of leaf rust used. No detailed analysis has been made of this interaction. According to Johnston (1934) "It seems probable that the mildew fungus splits some complex compounds, which the rust fungus is unable to use, into simpler compounds upon which it is able to subsist." Yet the mildew normally develops haustoria only in the epidermal cells of the wheat leaf, whereas the rust mycelium develops principally in the mesophyll; hence, the mildew fungus is apparently able to affect cell layers adjacent to those in which it is situated. Dillon Weston (1927) has given evidence that wheat infected by Bunt shows increased susceptibility to *Puccinia glumarum* under field conditions. This effect may be similar to the interaction of wheat rust and mildew mentioned above, although it is difficult to distinguish the influence of the pathogens on one another from the parallel influence of independent factors on infection by the two pathogens. The phenomenon of intercellular diffusion of substances produced in plant cells when invaded by a fungus is of great importance in connexion with the problem of acquired physiological immunity in plants.

On *a priori* grounds plants have often been considered to differ so fundamentally from animals as to be incapable of developing acquired immunity (Blackman, 1922,

1925); these differences are principally concerned with the circulatory system, the manner of growth, the reaction towards pathogens and the opportunities for sensitization. On the other hand, Carbone & Arnaudi (1930) and Chester (1933) have reviewed the subject comprehensively, and have concluded that acquired physiological immunity through the secretion of antibodies does occur among plants. Most of the evidence is controversial. For example, Ward (1902) in describing his experiments with the brome rust, *P. dispersa*, said: "All evidence points to the existence in the cells of the fungus of enzymes or toxins, or both, and in the cells of the host plant antitoxins or similar substances, as a decisive factor in infection or immunity, although I have as yet failed to isolate any such bodies." The subsequent discovery of the very numerous physiologic forms of cereal rusts has led some workers (Leach, 1919) to explain the differences between susceptibility and immunity on the basis of each physiologic form having highly specific food requirements, the specificity being, perhaps, between closely related stereoisomers of complex substances.

Bernard (1909*a, b*) concluded that the fungi involved in orchid mycorrhiza are parasites, the activity of which is controlled by the host reaction. The first rapid invasion of the host tissues by the fungus is soon checked by an acquired immunity which lasts for some time and prevents secondary infection. The host brings about this acquired immunity in part by absorbing or ingesting the invading hyphae, and in part by the production of antibodies, as was shown by the effect on the fungus in pure culture of substances secreted from infected orchid tubers (Bernard, 1911). These results were confirmed by Nobécourt (1923), Ramsbottom (1922) and Burges (1936). The proof of acquired immunity resulting in increased resistance to a pathogen through the production of antibodies is very difficult in plants, and has not yet been convincingly achieved. Frémont (1937), however, using an adaptation of the technique commonly applied in studies of animal immunology, concluded that far-reaching analogies exist between animal and plant cells in respect to acquired immunity from infectious diseases. The evidence in favour of the production of antibodies by diseased plant cells is still fragmentary, since antibodies appear often to be so closely bound up with living protoplasm that their properties cannot be investigated in non-living extracts. The interactions between virus diseases form an interesting comparison with the interactions of other pathogens on account of the ability of many viruses to spread systemically through the host plant with great rapidity.

Recent work has shown that plants infected with one strain of a plant virus may become immune from infection with other strains of the same virus. (Salaman, 1933; Kunkel, 1934; Price, 1935). The interactions occurring on the simultaneous inoculation of two viruses into the same host plant could be classified under several headings (Caldwell, 1935): one virus may induce either partial or complete immunity against the second virus; on the other hand, the disease resulting from the combination may be much more severe than that resulting from either virus alone; or the two viruses may multiply together, yet independently.

Several characteristics in common may be attributed to interactions between

organisms within or on the surface of host tissue. The interaction is usually restricted to a few organisms, often only two, and may vary in duration from a short parasitic or inhibitive interaction, to prolonged associative growth; in the latter instance, the disease symptoms resulting from the interaction may differ in degree or even in type from the symptoms caused by each pathogen alone. The interaction may be further complicated by reactions of the host tissues to the pathogens, and there is some evidence that plant cells are capable of being stimulated to the production of antibodies similar to those giving rise to acquired immunity in animals.

IV. INTERACTION ON ARTIFICIAL MEDIA

The phenomena associated with interaction on artificial media will be considered under three main headings:

- (1) Morphological modifications resulting from interaction.
- (2) Physiological analysis of interactions.
- (3) Comparison of interaction in culture and in nature.

(1) MORPHOLOGICAL MODIFICATIONS RESULTING FROM INTERACTION

Early experiments on the culture of fungi on artificial media showed that substances are produced by certain fungi which affect their subsequent growth. In an attempt to analyse the precise metabolic requirements of *Aspergillus niger*, Raulin (1869) weighed the mat of mycelium formed during three days on various liquid synthetic media; after removal of the mat the unsterilized medium was reinoculated and the crop of mycelium produced after a further three days was weighed; this procedure was repeated for a third three-day period. The first crop of mycelium was always the largest, although there was no apparent lack of nutrient. A number of investigations followed on the growth-affecting substances occurring in the filtrates from cultures of fungi and bacteria. However, the first descriptions of simultaneous interaction between two organisms did not occur until considerably later; Reinhardt in 1892 made numerous inoculations on artificial media with pairs of fungi side by side, and also with combinations of fungi and bacteria, and observed a variety of inhibitory effects. Subsequently a number of surveys have been made of the types of morphological interaction occurring between two fungi growing together on an artificial medium; these will be briefly discussed before considering the physiological basis of the phenomena.

Harder (1911) studied the reciprocal effects of pairs of fungi growing in petri dishes; he used a large number of combinations of Hyphomycetes with one another, and with Ascomycetes and Basidiomycetes, and of Hymenomycetes with Pyrenomycetes. Various grades of inhibition and overgrowth occurred, the result depending on each member of the combination. Porter (1924a) examined microscopically the zone of inhibition between two approaching colonies, and observed that one fungus exercised a dissolving effect at a distance on the hyphae of several other fungi, especially species of *Fusarium*. He also studied (1924b) the interactions of numerous pairs of organisms grown on cornmeal agar; using eighty cultures, mostly of fungi,

he described five principal types of interaction, of which the first represents compatibility, and the other four are progressively increasing grades of inhibition:

- (1) The two organisms intermingle mutually.
- (2) One organism overgrows and inhibits the other.
- (3) Slight mutual inhibition.
- (4) One organism grows around the other.
- (5) Mutual inhibition at considerable distance.

In a study of all the combinations in pairs of twelve citrus-rotting fungi grown on potato dextrose agar, Arrillaga (1935) recognized four of Porter's five types of interaction, and described three additional types of antagonism, depending on the nature of the inhibition zone.

Porter (1932) measured the growth rates of *Basisporium gallarum*, *Sclerotium rolfsii* and *Glomerella cingulata* on plates of potato dextrose agar, each of which had been thickly inoculated with a species of bacterium before being poured. Of ten bacteria tested in this way, two proved strongly inhibitory to the growth of these fungi, although mature colonies of *Basisporium gallarum* were able to grow over these bacterial colonies.

Endo (1931, 1932a, 1933) surveyed the interactions on beef bouillon agar of *Hypochnus centrifugus*, *H. sasakii* and *Sclerotium oryzae-sativae*, three rice pathogens important in Japan, with a large number of bacteria and other fungi. While many of the interactions were described as antagonistic, the three pathogenic fungi were indifferent to the presence of at least as many other organisms, and no general conclusions could be based on the results given.

Broadfoot (1933) investigated the interaction of sixty-six micro-organisms with *Ophiobolus graminis*, and found that two-thirds of them proved to be antagonistic on potato dextrose agar. He distinguished between compatibility and antagonism, and further subdivided the latter according to whether there was a neutral inhibition zone or merely a line of demarcation.

The systems of description used by Porter and Arrillaga attempt to cover all possible types of interaction between colonies of two organisms. However, the number of possible types may be indefinitely large, since the nature of an interaction depends on each member of the pair. The simpler method used by Broadfoot and by Brömmelhues (1935) of describing separately the effect of each interactant on the other appears to be more satisfactory. There are numerous other descriptions of changes in colony form induced by interaction (Machacek, 1928; Carter, 1935a), of which the majority range between indifference and inhibition. Only a few of the interactions cause acceleration of mycelial growth (Zeller & Schmitz, 1919; Arrillaga, 1935); yet the very nature of the technique of observing two colonies growing towards one another renders stimulation of growth very difficult to detect unless continuous measurements are made.

Interaction may affect other morphological characters apart from the form and rate of growth of a colony. Harder (1911) illustrates abnormal development of hyphae of *Coniophora* sp., caused by staling substances of a *Penicillium* sp., and abnormal conidiophores of a *Penicillium* sp., caused by staling substances from

Stereum purpureum. Porter (1924*b*) describes distortion of the hyphae of *Helminthosporium* sp., accompanied by the sudden appearance of bubble-like swellings, in the presence of a particular bacterium. A similar phenomenon was reported by Elliott (1917) with a species of *Alternaria*. In general, distortion is most marked when the inhibition is strongest. According to Brömmelhues (1935), the formation of a typical inhibition zone, which may be temporary or permanent, between two colonies is usually accompanied by an increase in the thickness of mycelium at the edge of the zone, and a thinning of the mycelium at other parts of the colony. Rosen & Shaw (1929) observed that *Sclerotium rolfsii*, when growing over a *Fusarium* colony, produced peripheral arborescent outgrowths similar to those formed by this fungus growing in the soil. Stevens & Ragle (1930) described dendritic, fibrous and ropy peripheral outgrowths formed by different races of *Sclerotium* in the presence of a number of other fungi. Endo (1931) confirmed this phenomenon when *Hypochnus centrifugus* was grown with antagonistic species of *Aspergillus* and *Penicillium*; and the author has seen *Sclerotinia fructigena* behave similarly when beside a colony of *Penicillium* species.

Anomalous pigmentation is frequently associated with interaction. Doebelt (1909) found that several fungi and bacteria favoured the production of pigment by a species of *Penicillium*, and even caused its production on media on which it was not normally formed. Beijerinck (1913) showed that the production of melanin from tyrosin resulted from the cooperative action of an actinomycete and a bacillus, each organism providing an essential enzyme. Further examples of anomalous pigmentation are discussed by Brömmelhues (1935) and Porter (1924*b*).

Interaction between colonies of the same fungus species have been widely studied in connexion with heterothallism (Cayley, 1931). A few cases of intra-specific aversion, i.e. inhibition, have been described among Ascomycetes. In the Phycomycetes Schmidt (1925) observed aversion between monospore mycelia of the same sex. Among Hymenomycetes aversion following failure of fusion in quadri-polar heterothallic forms is extremely rare; in bipolar species of this group aversion occurs sometimes between haplonts, and is usual between diplophase colonies, and may be accompanied by pigmentation along the lines of contact. In the haplophase aversion may or may not be correlated with the occurrence of heterothallic strains.

The formation of reproductive bodies by fungi through the effect of interaction has been frequently reported. Investigations of this phenomenon have, however, been primarily physiological, and will therefore be discussed later.

From the papers mentioned above it is apparent that the growth of a fungus may be unaffected or inhibited or stimulated in the presence of a second organism. Instances of mutual or one-sided inhibition have been commonly, and of stimulation only rarely, reported. Changes of the growth of a fungus, which may cause changes in colony shape, are often accompanied by other modifications of the characteristics of a colony, of the hyphae themselves, and of the development of reproductive organs.

(2) PHYSIOLOGICAL ANALYSIS OF INTERACTIONS

The physiological processes occurring when two fungi grow together in the same culture may conveniently be considered under the following headings:

- (a) Inhibition by simple metabolic products.
- (b) Inhibition by complex metabolic products.
- (c) Growth stimulation by complex metabolic products.
- (d) Reproduction stimulation by complex metabolic products.
- (e) Other factors affecting the nature of interaction.

These different aspects of the physiology of interaction will now be considered in turn.

(a) Inhibition by simple metabolic products

These products, commonly referred to as "staling substances", usually affect other organisms indirectly by causing changes in the medium, often through producing an unfavourable pH. They are simple, widely formed, and affect numerous organisms in greater or less degree; their production is dependent on the nature of the medium.

Nikitinsky (1904) considered that growth inhibition resulted from the accumulation of hydrogen or hydroxyl ions, except in the case of glucosides. This conclusion was not supported by Küster (1908) and Lutz (1909), who showed that the inhibitory properties were frequently destroyed by heating. Subsequent work has enabled a clearer distinction to be drawn between "staling substances", which will be considered very briefly here, and "inhibitory substances", which will be discussed in the next section.

Brown (1923) found that *Fusarium* species produced both "fixed alkali" and ammonia; on media, such as potato agar, from which ammonia was evolved, the growth of a colony of *Botrytis cinerea* was inhibited in all directions, because the whole surface of the medium absorbed the ammonia gas and became alkaline; on other media, colonies of *B. cinerea* were inhibited only on the side adjacent to *Fusarium*, as a result of the diffusion of "fixed alkali" from the latter. Pratt (1924) concluded that alkaline staling is due, in general, to the formation of bicarbonates from the carbon dioxide of respiration whenever there are free basic radicals. On the other hand, there are numerous examples of acid staling substances. Certain bacteria are able to exclude other organisms by producing large amounts of lactic, butyric and other acids. Similarly, *Aspergillus niger* stops almost all other growth but its own through the production of much citric acid; *Rhizopus* species produce the same effect with lactic acid, and yeasts with alcohol.

(b) Inhibition by complex metabolic products

Growth-inhibiting substances are mostly hypothetical, their existence having been postulated to explain the action of one organism, or of its culture filtrate, on the growth of another organism when the effect was not clearly due to a simple metabolic product of the type discussed above. Consequently, what is known of

the properties of these substances has been derived principally from studies on the filtrates from liquid cultures of organisms, and most attempts to isolate the substances themselves have met with no success.

Evidence for the production of inhibitory substances by bacteria was reported by De Freudenreich (1888), Nencki (1892) and others. Rahn (1906) and Eijkman (1906) considered that bacteria may produce two kinds of metabolic products, the first thermostable and favourable to growth, and the second thermolabile (at 60–100° C.) and unfavourable to the growth of bacteria. Pringsheim (1920) suggested that substances may be produced which are stimulatory at very low concentrations, but inhibitory at higher concentrations. Recent investigations on the antagonistic effects between bacteria have been principally concerned with pathogenic forms; a number of instances are discussed by Waksman (1937).

Gioelli (1933) attributed the mutual antagonism between *Penicillium digitatum* and *P. italicum* to the liberation by both organisms of toxins which were largely thermostable and capable of ultrafiltration. Carter (1935*b*) attributed the mutual growth inhibition between *Helminthosporium sativum* and a bacterium to an inhibitory substance, which diffused from staled agar into water, and survived autoclaving. Diffusion extracts of the substance exerted the same inhibitory effect on the growth of *H. inaequalis* as on *H. sativum*, but did not affect *Fusarium conglomerans*. Waksman & Foster (1937) showed that many soil organisms, including fungi, actinomycetes and bacteria, when grown on synthetic media, are capable of producing substances antagonistic to the growth of other soil organisms. They studied the antagonism of one species of *Actinomyces* against a variety of fungi and bacteria, as well as against other actinomycetes, and considered it to be caused by a substance which was rapidly destroyed by aeration or heat. Arrillaga (1935) investigated in detail the mutual growth inhibition occurring between *Diaporthe citri* and *Phytophthora parasitica* or *P. citrophthora*, in which the mycelium of the *Phytophthora* species was greatly modified. The inhibition was independent of pH changes, and was attributed to a chemical substance contained in the metabolic products of *Diaporthe citri*. This substance was constantly produced on a variety of different substrata, was diffusible and filterable, and withstood heating to 110° C.; hence it could not be an enzyme.

A definite substance, penicillin, has been found among the growth products of a group of *Penicillium* species (Fleming, 1929), and is able to suppress certain bacteria; it is rather unstable, and appears to be non-enzymatic. Reid (1933) confirmed the production of this substance by species of *Penicillium*; species of *Mucor* and *Aspergillus* did not form it. The substance was non-dialysable through a collodion membrane, resisted heating at 60–90° C. for short periods, and was unstable in storage. An inhibitory substance produced by another species of *Penicillium* (Reid, 1934, 1935) was relatively thermostable, but light, and gaseous oxygen, hydrogen or carbon dioxide prevented the formation of the substance, or caused its destruction; several enzymes were also present in the culture filtrate. The inhibitory substance was selective in action, affecting relatively few bacteria, of types apparently not closely related.

The nature of an inhibitor produced by *Diplodia zaeae*, which retarded growth of the mycelium of this species, has been investigated by Kent (1938). The action of the filtrate was not destroyed by oxidation, by correction of the hydrogen ion concentration, replacement of sugar in the cultural medium, moderate hydrolysis under acid or alkaline conditions, or by boiling for 1 hr. The above data, together with the alkaline reaction and insolubility in ether of the inhibitory material, suggest the presence of one or more complex nitrogen-containing compounds.

Harder (1911), Naegeli (1935) and others have shown that the amount of inhibitory substance produced varies with the age of the culture, being generally most abundant in old cultures. Waksman & Foster (1937) found the maximum production of inhibitory substance by a species of *Actinomyces* in cultures 7-18 days old. The inhibitory substance formed by *Diaporthe citri* (Arrillaga, 1935) did not appear in the culture until after 10 days, and was abundant after 20 days.

The specificity of the inhibitory substances differs widely. Some affect a variety of other organisms (Waksman & Foster, 1937; Arrillaga, 1935; Endo, 1931, 1932a, 1933). More often, however, each substance only affects one or a few species. Attempts to associate particular types of interaction with large groups of related organisms (Porter, 1924b) have met with little success; this is not surprising, since the type of interaction occurring between two organisms is, in general, a characteristic of both members of the pair. Hoyman (1938) studied the specificity of interaction between four species of *Aspergillus*. *A. niger* produced an inhibitory substance which did not affect its own growth, but reduced that of *A. terreus* approximately 90%, *A. ochraceous* 40% and *A. wentii* 50%. The substance produced by *A. terreus* inhibited its own growth as well as that of the other three species. The substance produced by *A. wentii* affected its own growth, and that of *A. terreus* and *A. ochraceous*, but not that of *A. niger*. *A. ochraceous* formed a substance which inhibited its own growth, but did not affect the other species. These results might be due to the different productivity and sensitivity of each species to some substance, such as citric acid.

Use of the specificity of inhibition interactions has been suggested as an aid in the identification of related species. Porter (1924b) distinguished two varieties of *Helminthosporium teres* by their interactions with several other organisms. Stevens & Ragle (1930) distinguished between eleven closely related races of *Sclerotium*, and even between strains of a species of *Glomerella*, by their growth interactions with fungi of several other genera. Coons & Strong (1931) drew up a scheme for identifying the species of *Fusarium* by their growth on media containing a series of graded concentrations of substances (mostly triphenylmethane dyes) inhibitory to growth.

Many inhibitory substances affect the spore germination of a species as well as its growth (Lutz, 1909; Küster, 1908; Machacek, 1928; Vasudeva, 1930; Satoh, 1931). The importance of this effect on germination among natural mixed populations would be considerable; in addition, the effect could prove useful in investigations on the properties of inhibitory substances.

The work of Raistrick and his collaborators (1931) has emphasized the ability of certain groups of fungi to synthesize characteristic series of complex compounds.

It seems that inhibitory substances comprise a number of miscellaneous types of metabolic product, each specific for one or several fungi, and all having the common property of inhibiting the growth of one or more organisms. Most of the inhibitory substances are complex, diffusible and filterable, non-volatile, and more or less heat stable, in consequence of which they are usually presumed to be non-enzymatic; within these broad limits there exists a great variety of properties, and it is clear that the substances do not conform to one type. Certain toxic chemicals simulate the action of inhibitory substances on a fungus colony (Porter, 1924*b*), and the stronger grades of inhibition by chemicals are accompanied by much distortion of the mycelium. This similarity emphasizes that intergradations probably occur between simple inhibition and toxic action. The production of a toxic (or lethal) substance by *Trichoderma lignorum* has been studied in detail, and merits some consideration; this substance contrasts with inhibitory substances in its mode of action, rate of formation, and stability, and in having been isolated and analysed.

The antagonistic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi (Weindling, 1932) is due to a lethal principle, which is responsible for the inhibition, death and disintegration of the opposing hyphae, whereas inhibitory substances merely prevent the growth of hyphae without killing them (in some cases death may take place subsequently from autolysis). Weindling (1934*b*) devised a uniform technique for examining the effects of various factors on the action of the lethal principle. The principle is characterized by its rapid deterioration: its effect is weakened by boiling, but not completely destroyed by even prolonged autoclaving. The effect decreases with increasing pH of the medium, apparently because of its faster decomposition at more acid values; under anaerobic conditions, however, its decomposition is independent of the reaction of the medium. The maximum rate of production of the lethal principle occurs two days after germination; this contrasts, presumably in part because of the rapid decomposition of the principle, with inhibitory substances, which usually occur in appreciable amounts only in old cultures. Weindling & Emerson (1936) isolated a crystalline substance to which the lethal properties could be attributed. This substance had the possible formula $C_{14}H_{16}N_2S_2O_4$; it decomposed readily, and, despite its nitrogen content, had no basic properties. Only by combining a clear knowledge of the mode of action of the substance on other fungi with chemical analysis of the nature of the substance can further progress be made in this field.

(c) Growth stimulation by complex metabolic products

The distinction between growth factors, which are essential for the growth of an organism, and stimulatory substances, which are not indispensable, cannot always be clearly drawn. A medium composed of simple salts and sugar was found adequate for the growth of yeast by Pasteur (1860), and for *Aspergillus niger* by Raulin (1869); Liebig (1871), however, failed to confirm Pasteur's results, and a bitter controversy developed. Wildiers (1901) found that yeast only grew well in a synthetic medium when large inocula were used, and he postulated that traces of "bios", a chemical substance essential for growth, were carried to the new medium if the inoculum was

sufficiently large. A review of this controversial subject by Tanner (1925) makes it clear that bios is essential for the normal growth of many yeasts. Bios is now known to consist of a number of components, including meso-inositol (Eastcott, 1928), vitamin B₁ and biotin; recently there have been distinguished a number of additional bios factors, which are briefly discussed by Kögl (1937).

Some organisms can grow well in a purely synthetic medium (Lumière, 1921; Goy, 1922); the growth of others is greatly improved by the addition of small amounts of growth factors (Linossier, 1919), although their effect naturally depends on the adequacy of the nutrient medium. Still other organisms cannot grow normally in the absence of growth factors; for example, Willaman (1920) showed that a growth factor, possibly vitamin B₁, was essential for the growth of *Sclerotinia cinerea*; likewise, Padwick (1936) found *Ophiobolus graminis* to be dependent on the presence of a growth factor of unknown nature, which could, however, be synthesized by certain bacteria. In general, inositol and vitamin B₁ appear to be two of the most important growth factors, and are essential for the normal development of many fungi and bacteria, not all of which can synthesize them (cf. the review by Peskett, 1933). According to Kögl and Fries (1937), *Polystictus adustus* is dependent on the addition of vitamin B₁ to a synthetic medium, and *Nematospora gossypii* requires biotin. Yet the two fungi were capable of developing together on a simple synthetic medium, the former fungus apparently producing the requisite biotin and the latter vitamin B₁. In his review of bacterial nutrition, Knight (1936) states that certain groups of bacteria are unable to synthesize substances essential to their growth, such as the respiratory co-enzyme required by *Rhizobia*, the sporogenes and staphylococcus growth factors, and the two factors required by *B. influenzae*. These special accessory substances can be synthesized by bacteria with simpler nutrient requirements, and are presumably supplied by them in the mixed associations occurring under natural conditions.

There are numerous references to stimulation of the growth of a fungus by extracts from the same or a different fungus, or from other micro-organisms. In most cases no clear analysis has been made of the nature or properties of the active substances in the various extracts. In two instances, however, both inhibitory and stimulatory substances have been obtained from the same extract, and their properties analysed in some detail.

Two kinds of substances, one retarding and the other accelerating growth and spore germination of *Aspergillus niger*, were formed in the culture liquid of *Ophiobolus miyabeanus* (Satoh, 1931). Their separation was simple, since the stimulatory substance passed a Chamberland filter (*F*), and the inhibitory substance did not. Moreover, the stimulatory substance was thermostable, while the inhibitory substance was thermolabile. The maximum effects of both substances were produced on *Aspergillus niger* during its first week of growth.

The culture filtrate from rose yeast, *Torula suganii*, was shown by Okunoki (1931) to contain both growth-inhibiting and growth-stimulating substances, as judged by their effects on various lower fungi; the substances affected only the growth and not the spore germination of these fungi, and had no effect on yeasts.

The inhibitory substance was heat-stable, non-volatile, unaffected by acids, but irreversibly destroyed at highly alkaline hydrogen-ion concentrations; it was readily soluble in hot ethyl alcohol, acetone, ether and chloroform, but not in carbon bisulphide or benzol; it was readily adsorbed on kaolin, Seitz filter or fungus mycelium at acid or weakly alkaline reactions. The growth stimulatory substance was heat-stable, and soluble in water, but not in alcohol or ether; it was not adsorbed by kaolin or Seitz filter. Hence, this substance could be separated from the inhibitory substance by the use of ether as a solvent, or kaolin or Seitz filter as adsorbents. The properties of this stimulatory substance agree with those described by Wildiers (1901) for bios, but the data are insufficient for a convincing comparison. The inhibitory substance only influenced young hyphae, and had no effect on conidial germination or on mature mycelium. Consequently, the inhibitory effect diminished in long-continued cultures, finally leaving only the stimulatory effect apparent.

(d) *Reproduction stimulation by complex metabolic products*

As early as 1903 Molliard observed that a species of *Aspergillus* growing on carrot produced apothecia only when the cultures were contaminated with a bacterium. Sartory (1912, 1920) reported that the presence of a bacterium was necessary for the sporulation of a yeast and for the production of perithecia by a species of *Aspergillus*. The influence of one fungus on the reproduction of another was first described by Heald & Pool (1909), who showed that *Melanospora pampeana*, which did not normally form perithecia in culture, produced them very abundantly in the presence of *Basisporium gallarum*; an effect almost as great was caused by *Fusarium moniliforme*, a small effect by *F. culmorum*, and no effect by other species tried. Since there was no union of hyphae of the interacting fungi, the property was attributed to a special substance, which was found to resist sterilization at 110° C. Monoconidial cultures of *Thielavia basicola* produced perithecia in culture only in the presence of certain other fungi (McCormick, 1925); the extracts of these fungi retained their stimulatory properties when sterilized by passage through a Berkefeld filter, but were rendered ineffective when heated to 100° C. for 20 minutes. Similarly, Wilson (1927) found that the ability of a mycelial extract of *Penicillium* species to stimulate the formation of perithecia by *Venturia inaequalis* was destroyed by autoclaving. Extracts from *Penicillium glaucum* stimulated the production of conidia by several species (Sibilia, 1929), although no stimulation occurred in the presence of colonies of *P. glaucum*. Mehrlich (1935) found that a non-sterile soil percolate greatly favoured the production of zoosporangia by *Phytophthora cinnamoni*; the zoosporangia formed in this way were slightly atypical. The interesting observation was made by Koch (1934b) that *Dibotryon morbosum* growing on potato glucose agar produced chlamydospores only near certain bacteria, or *Cephalothecium roseum*, or on the addition of a drop of sulphuric acid or copper sulphate.

Arrillaga (1935) investigated a substance formed by *Diaporthe citri* which both inhibited the growth, and also stimulated the development of reproductive structures, by *Phytophthora citrophthora*. This difference in the effects of one substance on

two physiological processes of the same fungus is striking, although it is known that the conditions favouring the two processes are usually dissimilar; substances formed by certain other fungi favour growth as well as reproductive activity (Heald & Pool, 1909).

Asthana and Hawker (1936) showed that the sporulation of *Melanospora destruens* and other Ascomycetes is stimulated in the presence of certain other organisms, and by extracts from the cultures of these organisms. The influence of the interactants on *M. destruens* was attributed to reduction in concentration of nutrients, production of inhibitory substances, and to the production of one or more substances which stimulate perithecial formation, and which are produced but slowly by *M. destruens* itself. A crude extract of lentils was also effective in stimulating sporulation of *M. destruens* and a number of other fungi (Hawker, 1936). The active substances from lentil extract and from fungal products had similar effects on the sporulation of *M. destruens*, *Sordaria fimicola*, *Rosellinia necatrix* and *Zygorhynchus molleri*. The fungal preparations, however, were poor in inositol; and, while some fungi required both fractions of the active extract for sporulation, the inositol fraction proved unnecessary for *Melanospora destruens* and several other fungi.

(e) Other factors affecting the nature of interaction

Liesegang (1920) is one of the very few who have attributed interaction, as a whole, to exhaustion of nutrients. The available evidence shows that competition for nutrients is likely to be of importance only in the absence of special substances exerting pronounced control over growth processes; nevertheless, the production of such substances may depend in certain circumstances on the nature of the medium (Weindling, 1932; Broadfoot, 1933).

When the processes by which fungi influence the growth of one another are closely balanced, and there is no clear dominance, a minor factor may exert a controlling influence on the final result. Temperature may be of importance under certain conditions (Gioelli, 1933; Savastano & Fawcett, 1929); or the result may vary with different relative sizes of inocula of the organisms (Machacek, 1928; Porter, 1924b). The nature of the interaction may also depend on the relative times at which the interactants start growing (Vasudeva, 1930). These factors may have an important influence on the succession of fungi (Cook, 1924), a subject which has received little attention as yet; in this connection Fraser (1937) has made an excellent study of the physiological factors underlying the distribution of sooty-mould fungi.

(3) COMPARISON OF INTERACTION IN CULTURE AND IN NATURE

The type of interaction occurring between fungi in artificial culture sometimes differs greatly from that occurring under natural conditions. A number of instances of such differences were observed by Machacek (1928) and Endo (1931, etc.). *Penicillium digitatum* and *P. italicum* have been shown (Gioelli, 1933) to be mutually antagonistic on oranges or lemons, but not on artificial media. In his study of the interaction of numerous soil micro-organisms with *Ophiobolus graminis*, Broadfoot

(1933) found that, of twenty-one organisms controlling the virulence of *O. graminis* in the soil, fifteen were also antagonistic on potato dextrose agar; moreover, of forty-five organisms giving only moderate or no control in the soil, twenty-eight proved to be antagonistic in culture; and, further, *Typhula graminum*, which gave moderate control in the soil, was antagonistic on Molisch's medium, but not on potato dextrose agar. Hence, extreme caution must be observed in attempting to generalize about interactions in nature on the basis of results obtained with artificial media. The use of synthetic media should rather be regarded as a technique for elucidating one at a time the physiological factors which would presumably lead to a complete understanding of interactions under natural conditions.

V. SUMMARY AND CONCLUSIONS

The numerous instances of interaction between fungi (and other micro-organisms) can be classified satisfactorily into three groups on the basis of the substrate in which the organisms are growing. The principal types of interaction may be distinguished as occurring (a) in the soil, (b) on or in a host plant, and (c) on artificial media.

The soil interactions may be regarded as taking place between micro-organisms in an independent medium in which their metabolic products can readily diffuse. Interaction between soil micro-organisms is always highly complex since it concerns to a greater or less degree all members of the soil microflora, of which the qualitative and quantitative constitution is rapidly altered with changes in external conditions. Moreover, the numerous micro-organisms may interact with one another in different ways; on the one hand, activity may be depressed by competition for nutrients, by changes in the reaction of the medium, or by the secretion of specific inhibitory or toxic substances; on the other hand, interaction may be beneficial, through the interlocking of metabolic requirements, or the secretion of stimulatory substances. The part played by each process cannot usually be evaluated, but the equilibrium value of the whole complex is represented at any one time by the composition of the soil microflora; and the abundance of any particular organism may be viewed as just one expression of this momentary equilibrium.

A soil-borne pathogen must be able to survive interaction with the saprophytic microflora before it can parasitize the roots of a host plant. The importance of this factor in determining the occurrence of certain soil-borne diseases, e.g. foot-rot of wheat caused by *Ophiobolus graminis*, is now realized, and attempts are being made to increase antagonism by cultural practices.

Interactions occurring on host plants generally involve only a small number of organisms. The host tissue, which forms the substrate for interaction, is relatively constant and homogeneous, though complex, and does not allow the ready diffusion of metabolic products by which organisms could affect one another at a distance. Several concurrent physiological processes may be concerned in interactions occurring on host tissue, as in interactions between soil micro-organisms; the outcome of an interaction will represent the equilibrium balance of this complex

of processes, and will be affected by the incidence of changing external factors on each process. On the basis of the duration and intensity of interaction, examples could be placed in a series ranging from direct parasitism, through successional disease, to continued growth in association. That is, the interaction may be quickly over, allowing one fungus complete dominance (usually through the action of a potent metabolic substance, or because one organism is in a highly susceptible phase); or two fungi may grow together in the same place, and yet develop independently if their spheres of physiological activity do not overlap. In many instances one or other of these processes is known to be of particular importance. A complete understanding of any one interaction, however, must be based on a thorough knowledge of the physiology of each organism concerned, and especially of their enzyme complements and metabolic products. The reaction of the host to the interacting organisms is a factor about which present knowledge is mostly conjectural.

The interaction of fungi on artificial media has been principally studied in two ways: by growing two organisms side by side, and by adding the culture filtrate from the growth of one organism to the medium upon which a second organism is to be grown. Two fungi grown side by side frequently show inhibition of growth, either one-sided or reciprocal, along the common axis; this inhibition may be accompanied by profound changes of colony form and of the morphology of the mycelium. Instances of growth stimulation are less readily detected, and have only occasionally been reported; however, interaction is known to stimulate reproduction of certain fungi.

Experiments on artificial media have elucidated several physiological processes concerned in interaction between micro-organisms. Inhibition may be caused by specialized metabolic products, of complex and unknown chemical constitution, more or less specific in action, and effective at very low concentrations; one toxic fungal product, with properties somewhat similar to these inhibitory substances, has been isolated and assigned a provisional formula. Another group of metabolic products causing inhibition comprises simple non-specific substances, commonly formed in considerable quantities, which often act by changing the reaction of the medium.

The substances responsible for this stimulation of growth and reproduction of fungi are little understood, but appear to be complex, more or less specific in formation and in action, and effective at very low concentrations. It seems that bios and vitamin B₁ may be important stimulatory substances for a number of fungi and bacteria.

A number of other factors, such as competition for nutrients, effect of external conditions, and relative sizes of the inocula, are apparently of minor importance except when the previously mentioned processes of interaction do not have a decisive effect.

The type of interaction occurring between two organisms on artificial media may be quite different from that occurring between the same two organisms in nature. The use of synthetic media should be recognized as a technique for

elucidating one at a time the physiological factors which are concerned in interactions in culture; presumably the sum total of these would lead to an understanding of interactions under natural conditions.

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ADDENDUM

Two extensive reviews of the literature on interaction between fungi have been published since this article was written. The first, entitled "Competition among Fungi," is by C. L. Porter and J. C. Carter (*Rev. Bot.* **4**, 165-182, 1938); and the second, on "Association Effects of Fungi", is by R. Weindling (*Rev. Bot.* **4**, 475-96, 1938).

THE MECHANICS OF SEA URCHIN DEVELOPMENT, STUDIED BY OPERATIVE METHODS

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(Received 21 June 1938)

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I. INTRODUCTION

SINCE the discovery by Hans Driesch (1891) that a harmonic sea urchin larva can arise from an isolated $1/2$ -blastomere, and thus that a part of an egg can develop into a whole organism, the eggs and larvae of sea urchins have been subject to numerous investigations regarding determination. The scope of this article is to give a short review of our present knowledge of determination in the sea urchin, as studied by means of local vital staining and operative methods, such as fragmentation and transplantation, particular prominence being given to the author's own work in the field. As the limited space does not allow either of a full treatment of the other groups of the echinoderms, or of a detailed presentation of the numerous studies on metabolism, on the effect of chemical agents, on centrifugal force, etc., only a few of those results will be mentioned when necessary for comparison. Some of the older results, erroneous or incomplete, will be mentioned only very briefly, it being necessary to concentrate upon the more definite results of to-day. A more complete review of the literature is to be found in Hörstadius (1928*b*, 1935), Schleip (1929), and Lindahl (1936*a*). In this article the author's name will be quoted as H-s.

II. THE EARLY DEVELOPMENT OF THE SEA URCHIN

As our type we choose *Paracentrotus* (*Strongylocentrotus*) *lividus* Lk., which has been much used for experiments. The egg of this sea urchin is particularly suitable for experiments as, thanks to a pigment band, the polarity can be already recognized in the mature egg (Selenka, 1883; Boveri, 1901*a*, *b*). The pigment band (in eggs

from some females the band may not be visible) occupies the vegetative half of the mature egg, except for an unpigmented polar cap. In other species the polarity can only be traced by the location of the polar bodies and the micropyle. The pigment band is a better landmark for orientation. Boveri (1901 *a*, *b*) thought that the micropyle, which marks the animal pole, also indicates the point of attachment in the ovary, but Jenkinson (1911 *b*) and Lindahl (1932 *a*) state that the micropyle is antipolar to the point of attachment.

The first two cleavage furrows are meridional, the third equatorial, dividing the egg into eight equal blastomeres, that is if the third furrow is strictly equatorial (Fig. 1 A-C). Sometimes the third furrow cuts through farther toward the vegetative pole, and as a consequence the four animal blastomeres will be larger than the vegetative ones and will thus contain a part of the material really belonging to the vegetative half (subequatorial eggs, H-s, 1935, p. 262). The next cleavage is unequal, giving one ring of eight animal mesomeres, four large, pigmented macromeres, and, at the vegetative pole, four small, unpigmented micromeres (Fig. 1 D). In the 32-cell stage the animal half consists of two rings of eight cells each, which are designated as an_1 and an_2 (H-s, 1931, 1935). In the 64-cell stage the macromeres have divided into two tiers of eight cells, each of which we call veg_1 and veg_2 (Fig. 1 F). We may thus divide the egg into five layers: an_1 , an_2 , veg_1 , veg_2 , and the micromeres.

The blastula soon acquires active cilia. At the animal pole we find an apical tuft of long, stiff cilia (Fig. 1 H). Even before gastrulation, the material derived from the micromeres (Boveri, 1901 *a*, *b*; H-s, 1935, p. 279) migrates into the blastocoele as the primary mesenchyme, which will later give rise to the skeleton (Fig. 1 I). Boveri (1901 *a*) concluded from his studies of the pigment band that the entire vegetative half (except of course the micromere material which has already formed the primary mesenchyme), that is $veg_1 + veg_2$, invaginates to form the archenteron, and this view was supported by v. Ubisch (1925 *a*) by vital staining (method of Vogt). Many experimental results were, however, inexplicable on the assumption of an ecto-entodermal boundary an_2-veg_1 , and it was found (H-s, 1931, 1935, 1936 *b*) by vital staining that only veg_2 invaginates to form the archenteron. At the same time the prospective significance of the layers an_1 , an_2 , and veg_1 were established (Fig. 1 K-N): an_1 forms the most animal part of the larva, giving rise to the apical tuft, the oral lobe, the oral arms, and also the stomodaeum. veg_1 constitutes the ectoderm of the anal side of the pluteus, extending a little also on to the right and left sides. The statements as to the position of the lines an_2-veg_1 and veg_1-veg_2 were confirmed by v. Ubisch (1933 *c*) by new vital staining experiments. By counting the nuclei in the gastrula Driesch (1902 *b*) and Schmidt (1904) had already arrived at the same result, namely, that only about one-fourth or one-fifth of the material of the blastula invaginates, but the statements of Boveri and v. Ubisch had been generally accepted.

The vital staining of the presumptive archenteron not only showed that a much smaller amount of material is invaginated than was generally believed, but also demonstrated a mode of invagination essentially different from that previously

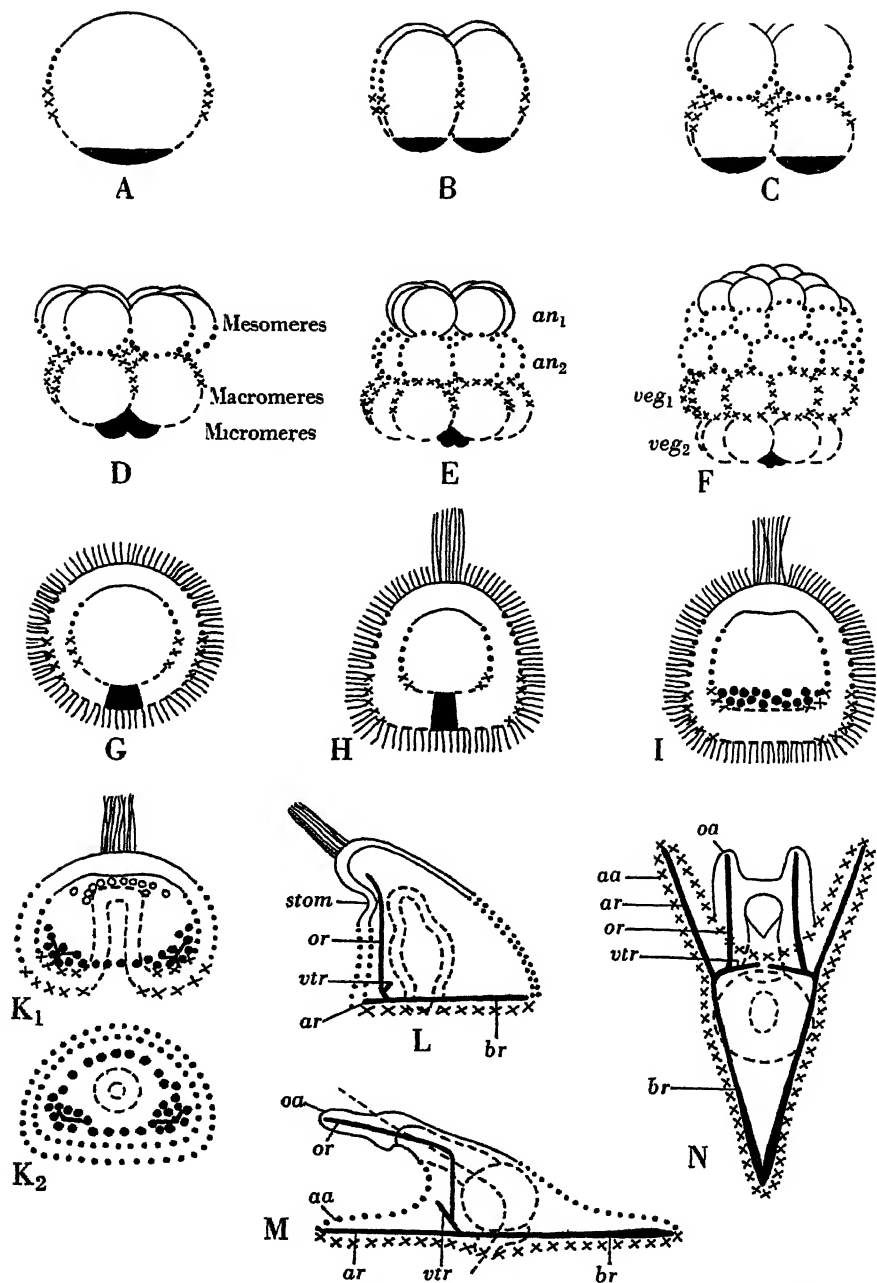


Fig. 1. Diagram of the normal development of *Paracentrotus*. Indication of the layers: an_1 , continuous lines; an_2 , dotted; veg_1 , crosses; veg_2 , broken lines; micromeres, black. A, uncleaved egg. B, 4-cell stage. C, 8-cell stage. D, 16-cell stage. E, 32-cell stage. F, 64-cell stage. G, young blastula. H, later blastula, with apical organ, before the formation of the primary mesenchyme. I, blastula after the formation of the primary mesenchyme. K_1 , gastrula: secondary mesenchyme and the two triradiate spicules formed. K_2 , transverse optical section of the same gastrula; bilateral symmetry established. L, the so-called prism stage; stomodaeum invaginating. M, pluteus larva from the left side; the broken line indicates the position of the egg axis. N, pluteus from the anal side. aa , anal arm; ar , anal rod; br , body rod; oa , oral arm; or , oral rod; $stom$, stomodaeum; vtr , ventral transverse rod. (H-s, 1935.)

described. According to the old view (Herbst, 1893, 1896; Morgan, 1895c; Schmidt, 1904; Garbowski, 1905b) a vegetative plate, the tip of the archenteron, is lifted up and moved into the blastocoele by gradual invagination of more and more material, which would thus form the sides of the archenteron, and because of this turning in of a large amount of material the volume of the blastula would decrease during gastrulation. The vital staining of the presumptive archenteron (*veg*₂) showed, however, that from the beginning the invagination comprises the entire material that will gastrulate. The invagination is brought about by a fairly uniform stretching in animal-vegetative direction of the whole *veg*₂ disk. This mode of gastrulation was further proved by staining a single *veg*₂ cell. The volume of the blastula does not decrease; on the contrary, it is increased during gastrulation owing to a stretching, in all directions, of the ectoderm (H-s, 1935, pp. 269-78, 1936b).

At the tip of the archenteron the secondary mesenchyme and the coelom are budded off. At this stage we find the first traces of bilateral symmetry, as one side of the gastrula is flattened and thickened to develop into the oral field, surrounded by the ciliated band, the most animal part of which is thickened and corresponds to the plate of the apical tuft in the blastula (Wimperschopfplatte, Lindahl, 1936a, p. 184). On the flattened, ventral side, we also find two triradiate spicules, the first rudiments of the skeleton (Fig. 1 K₂). From the oral field an ingrowth of the ectoderm forms the stomodaeum, which joins the tip of the archenteron (Fig. 1 L, M). The archenteron is divided into oesophagus, stomach, and intestine.

Two oral (anterolateral) and two anal (postoral) arms are formed, supported by skeletal rods (Fig. 1 M, N, *oa*, *aa*, *or*, *ar*). The position of the skeleton-forming cells (primary mesenchyme) is determined by the ectoderm (Herbst, 1893, p. 193, 1896; Driesch, 1896b; Schmidt, 1904; Runnström, 1928a, b, 1929, 1931; H-s, 1928b, p. 39). On the other hand, the skeleton or the skeleton-forming cells exert an influence upon the ectoderm, which influence (*formativer Reiz*) is necessary for the formation of the arms (Herbst, 1892, p. 456, 1893, pp. 193, 206, 1896, 1897), although the arm has to be predetermined in the ectoderm before the skeleton rod reaches the ectoderm (Runnström, 1917, 1928a, b, 1929). Thus an interaction between ectoderm and skeleton is necessary for a normal arm to result. Rudiments of arms may appear without any skeleton, but they do not develop far (Jenkinson, 1911a; MacBride, 1914; Runnström, 1915, 1931, p. 288; v. Ubisch, 1933c; H-s, 1935, p. 50). Arms do not, however, always grow out where a skeleton rod reaches the ectoderm (Runnström, 1929, p. 131; v. Ubisch, 1931a, b, 1932a, 1933c). If a rod is missing, another rod may grow towards its place and act as its substitute (Runnström, 1928b, 1929, 1931; H-s, 1935, p. 430).

The position of the egg axis in the fully differentiated pluteus larva is indicated by the broken line in Fig. 1 M. The animal pole of the gastrula corresponds to the front edge of the oral lobe (the apical plate) between the oral arms. The oral field occupies the greater part of the ventral side. The pointed posterior part of the body, with the two long, thickened body rods (*br*), is situated on the dorsal side of the pluteus (Runnström, 1928b; H-s, 1928b, p. 67).

Many other species have been used for experiments, e.g. *Psammechinus* (*Parechinus*, *Echinus*) *miliaris* and *Ps. microtuberculatus*, both having skeletons of the same simple type as *Paracentrotus*, furthermore *Sphaerechinus granularis*, *Arbacia pustulosa* and *A. punctulata*, *Lytechinus variegatus*, *Echinocardium cordatum*, *Echinocyamus pusillus*, and others, all with a more complex skeleton, some of the rods being of the lattice type (fenestrated). In what follows the species used will be mentioned only in a few cases, when the point is of special interest.

To express the constitution of a larva from which material has been removed I shall use formulae (H-s, 1931, 1935) which refer to the normal 16-cell stage: $8+4+4$ (8 mesomeres, 4 macromeres, 4 micromeres). Thus a larva from which the an_1 ring has been removed is characterized as an_2+4+4 , a larva $8+veg_1+0$ is devoid of both the veg_2 ring and the micromeres, and so on.

III. METHODS

Sea urchin eggs are easy to fertilize artificially. The jelly and the fertilization membrane are removed by shaking, or, better, by squirting the eggs through a fine pipette (Plough, 1927). Formerly blastomeres were isolated by shaking, without (Fiedler, 1891; Driesch, 1891) or with (Driesch, 1902b) previous treatment with calcium-free sea water, which loosens the connexion between the blastomeres (Herbst, 1900). Zoja (1895) cut cleavage stages, Jenkinson (1911a) blastulae and young gastrulae with fine knives. Spemann (1906) recommended fine glass needles for operations on sea urchin larvae, and such needles are now usually employed (v. Ubisch, 1925b; H-s, 1925b). Vogt's method of local vital staining with small pieces of agar was first applied to sea urchins by v. Ubisch (1925a). Lindahl (1932b, p. 301) encloses the agar in a very fine pipette, which allows of extremely small marks being made; for instance, a single cell of the 64-cell stage can be stained (H-s, 1936b). For transplantations the parts to be fused are placed, after treatment with calcium-free sea water, one on top of the other in a small depression made in a thin plate of celluloid, and a small glass ball is put for a while on top of the upper piece, to give the necessary pressure (H-s, 1928b, pp. 5-9, where also the literature on methods is more fully reviewed).

IV. DETERMINATION OF CLEAVAGE

Selenka (1883) and Boveri (1901 a, b) stated that the first two cleavage furrows in the *Paracentrotus* egg lie meridionally. But their position may be different if, before cleavage, the egg is markedly stretched (Boveri, 1901b) or centrifuged (*Arbacia*, Lyon, 1906 a, b; Morgan & Lyon, 1907; Morgan, 1909; Morgan & Spooner, 1909).

As to the problem whether the position of the first furrow coincides with the entrance point of the sperm, observations vary. According to Selenka (1878), Wilson & Matthews (1895), Boveri (1901b), and Runnström (1926) the entrance point of the sperm and the first furrow generally coincide, whereas the observations of Garbowski (1905b) and Boveri (in Heffner, 1908) indicated that this is not so.

Vital staining marks made opposite the point of entrance of the sperm were found to lie in any position relative to the first furrow (H-s, 1927, 1928b).

Boveri (1901b) assumed that changes in the cytoplasm cause the spindle of the third division to lie in an animal-vegetative direction (the spindles of the first two cleavages are normally confined to the equatorial plane, see above), and that further changes in the cytoplasm determine the position of the spindles at the formation of the mesomeres and the macro- and micromeres. This view was confirmed by several observations (Boveri, 1902, 1903, 1905, 1907, 1910b; Painter, 1915). The formation of micromeres is, however, due not only to the position of the spindles, but also to a special character of the vegetative cytoplasm. Morgan (1894, *Arbacia*) and Boveri (1901a, b, *Paracentrotus*) observed that the cytoplasm at the vegetative pole has a particular structure. Driesch (1896d) assumed chemical differences, and Morgan (1895b) presumed the presence of a micromere region already in the undivided egg, since eggs which, after shaking, cleaved directly into three blastomeres, later on gave six micromeres. Morgan & Spooner (1909) concluded from centrifuge experiments that a larger region than the normal micromere-forming field is capable of producing micromeres, and Hörstadius (1927, 1928b) showed, by the removal of fragments of different size, that the material capable of forming micromeres reaches (although with decreasing intensity) higher up than the unpigmented polar cap—up to about the middle of the pigmented region.

Sometimes whole eggs have been found to segment as fragments (Driesch, 1893a, 1906; Morgan, 1894; Zoja, 1895; Boveri, 1905, 1910b; H-s, 1927, 1928b). Driesch observed that eggs in diluted sea water often form micromeres already in the 8-cell stage (*vorzeitige Mikromeren*), thus cleaving as a vegetative half (Fig. 2B₄). He speaks (1896d, 1903b, 1906) of an "anachronism of the furrows". Boveri (1905, 1910b) obtained 1/2- and 1/4-cleavage after shaking the eggs: once or twice only monasters were formed, that is to say there were one or two nuclear division cycles without division of the cytoplasm. This confirmed the hypothesis of Boveri (1905, p. 17, 1907, 1910b) that the position of the spindles in different planes (first equatorial, then animal-vegetative) at different times is dependent upon a determination process initiated at fertilization and independent of the cleavage of the cytoplasm. This was further shown by Painter (1915), who, by means of phenylurethane, inhibited the nuclear divisions for some time, and afterwards obtained several types of partial cleavage. The appearance of abnormally early micromeres in diluted sea water is associated with a delayed nuclear division (Driesch, 1893a, 1906; Konopacki, 1918; Paspaleff, 1927; H-s, 1927, 1928b). Hörstadius explained the types in diluted sea water—also those with only two micromeres and the cleavage axis oblique to the egg axis—as being a result of a delayed cleavage in relation to the progressive determination in the cytoplasm (Fig. 2).

Driesch (1896d) isolated pieces of unfertilized eggs (*Psammechinus*) by shaking and obtained in some cases whole, in some partial cleavage. Driesch (1896d, 1898b) held the view that the differences are due to a different faculty of regulation, i.e. a rearrangement of the particles. Boveri (1901b) observed that meridional and vegetative fragments of *Paracentrotus* eggs segmented as whole eggs, while animal

fragments (unpigmented) gave blastomeres of equal size, as the animal half normally does. Hörstadius (1927, 1928*b*) confirmed these observations on fragments isolated with glass needles and oriented by aid of the pigment band (*Paracentrotus*). The lack of micromeres in animal fragments is due, not to differences in regulation, but

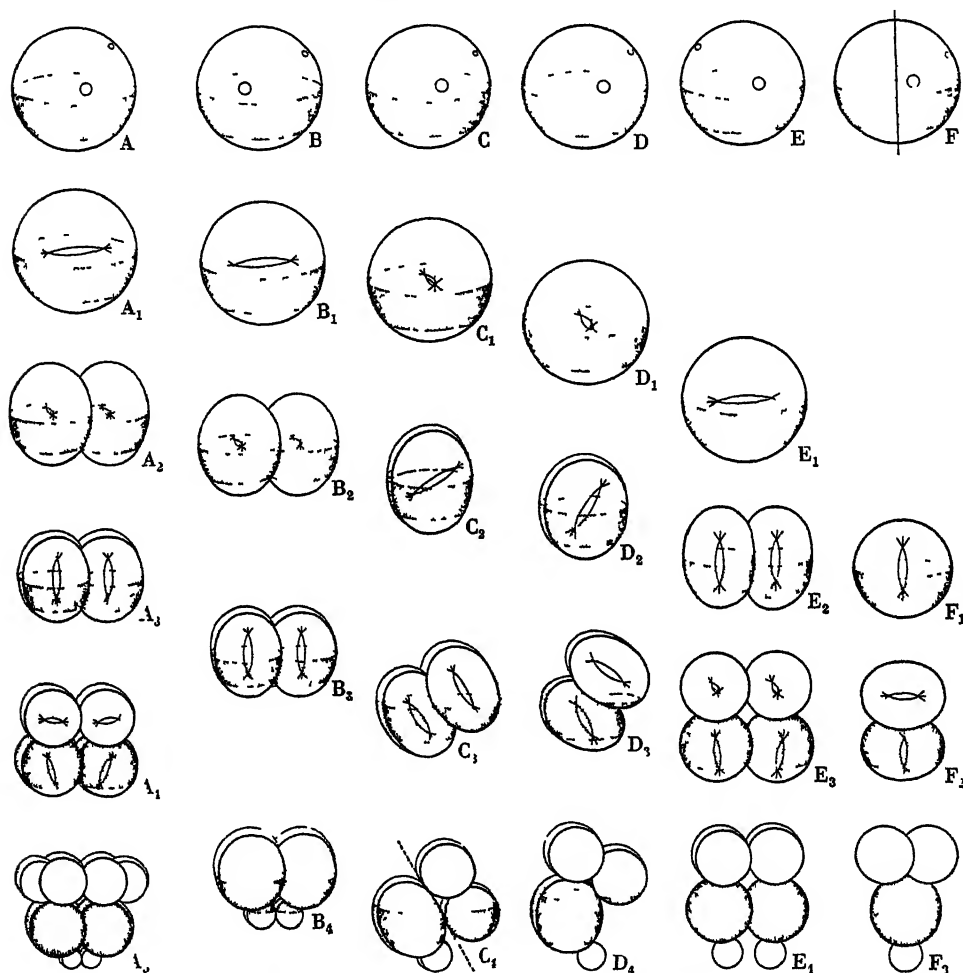


Fig. 2. Diagram of cleavage in *Paracentrotus*. A-A₅, normal cleavage. B-F, cleavage delayed in relation to the process of determination, affecting the position of the cleavages spindles and causing the activation of the micromere cytoplasm. The delay was caused by hypotonic sea water or by shaking. The normal cleavage A-A₅ serves as a time scale. All stages oriented with vertical egg axis. (H-s, 1928*b*.)

to lack of micromere-forming material (Boveri, H-s). The fact that a vegetative half does not form micromeres in the 8-cell stage, when the spindles occupy nearly the same position as at the division leading to the normal 16-cell stage, indicates that the micromere material is not activated as such until a time corresponding to the normal micromere formation (H-s, 1927, 1928*b*). Lindahl (1936*a*, 1937) assumes a local condensation of the cortical cytoplasm at the vegetative pole.

Thus the factors determining the cleavage type of the 16-cell stage seem to be progressive changes in the cytoplasm, causing spindles formed a certain time after fertilization to lie in a certain direction, the presence in the vegetative part of the egg of a region of micromere-forming material, and the activation of that material a certain time after fertilization (see Fig. 2).

It is strictly in conformity with this analysis that isolated blastomeres ($1/2$ -, $1/4$ -, or groups of $1/8$ -blastomeres) show a partial cleavage as regards the number of meso-, macro-, and micromeres (Driesch, 1891, 1892, 1895*b*, 1900*a*, 1903*b*; Fiedler, 1891; Zoja, 1895; and others). The fragments soon round up to form a closed blastula.

Fragments of fertilized eggs sometimes show whole, sometimes partial, cleavage (Driesch, 1896*d*; H-s, 1927, 1928*b*). Boveri (1901*b*) opposed Driesch's view that the difference is due to a different faculty of regulation. When fragments are identical as to the stratification (meridional fragments) Boveri assumed whole cleavage to occur when the surface of the fragment all round is of the same character as in the normal egg, while half cleavage would appear when one side of the fragment is devoid of the normal surface layer. But the following interpretation of the cleavage of fragments of fertilized eggs is more probable. Animal halves segment equally, vegetative halves show whole cleavage because of the absence or presence respectively of micromere-forming cytoplasm. The frequency of $1/1$ -, $3/4$ -, and $1/2$ -cleavage of meridional halves depends upon the time of isolation: fragments isolated shortly after fertilization give whole cleavage; those isolated shortly before first division give half cleavage, in the period between they give intermediate stages (H-s, 1927, 1928*b*, pp. 17, 141). Thus the type of cleavage depends upon the time of isolation in relation to the determination process in the cytoplasm initiated at fertilization.

Not all meridional and vegetative fragments show micromeres, for the micromere formation easily becomes inhibited, e.g. by heat or pressure (Driesch, 1892), as a result of fragmentation (Zoja, 1895; H-s, 1928*b*, p. 18), of stretching (Boveri, 1901*b*), or of shaking (H-s, 1928*b*, p. 124).

Harnly (1926) concluded that the micromere material in the unfertilized egg of *Arbacia* is situated between the nucleus and the centre of the egg, but it has since been found that the conditions in *Arbacia* are the same as in *Paracentrotus* (H-s, 1937). Taylor & Tennent (1924), Taylor *et al.* (1926) and Tennent *et al.* (1929) state that there is hardly any localization of micromere material in *Lytechinus* (*Toxopneustes*) *variegatus*. In fragments a new axis is said to be established perpendicular to the cut surface, and the micromeres appear always at the cut side. As the three authors made some experiments also on *Arbacia*, and their results do not agree with a renewed investigation of that form (H-s, 1937), which seems to confirm the results on *Paracentrotus*, further investigations on *Lytechinus* are desirable.

A whole larva may arise from an isolated $1/2$ -blastomere, although segmenting as a half (Driesch, 1891, 1892, 1900*a*, 1903*b*, and others). We may also get a typical development, although owing to pressure the nuclei are atypically distributed, and the furrows appear in atypical order or place (Driesch, 1892, 1893*b*;

Morgan, 1894); and likewise if the furrows are displaced owing to centrifuging, or stretching, or shaking (see above). These results indicate that the differentiation of the sea urchin egg is independent of the type of cleavage (Boveri, 1889, 1895; Driesch, 1896*d*, 1898*a*; and others).

V. DETERMINATION OF THE POSITION OF THE AXES

It has been mentioned above (p. 133) that the egg axis (the animal-vegetative axis) is already laid down in the ovary, the animal pole, where we find the micropyle and the polar bodies, being antipolar to the point of attachment. The apical tuft appears at the animal pole, the primary mesenchyme and the archenteron at the vegetative. In isolation experiments animal fragments give only ectoderm, vegetative fragments give both ecto- and endoderm. The egg axis is also characterized by a reduction gradient (Child, 1936*a*) and a susceptibility gradient (Child, 1915, 1916*a, b*), and by different types of metabolism (Runnström, 1928*b*, 1933, 1935; Lindahl, 1933, 1934, 1935, 1936*a*, 1938; Lindahl & Stordahl, 1937*b*; Lindahl & Öhman, 1938).

The egg axis is more stable than the dorso-ventral and the right-left axes. A moderate stretching (Boveri, 1901*b*; Lindahl, 1932*b*, 1936*a*) or centrifuging (Lyon, 1906*a, b*; Morgan & Lyon, 1907; Morgan & Spooner, 1909; Morgan, 1909, 1910) does not change its position, but a marked stretching or a constriction may shift its position or divide it into two, the vegetative pole being more stable than the animal (Lindahl, 1932*b*; H-s, 1938).

The polarity of fragments of unfertilized eggs of *Paracentrotus*, studied by means of the pigment band, was found to remain unchanged, both as regards cleavage (Boveri, 1901*b*, p. 157; H-s, 1928*b*, p. 15) and differentiation (Boveri, 1901*b*, p. 158; H-s, 1936*b*, p. 53). The statement by Tennent *et al.* (1929) that in fragments of *Lytechinus* the axis is at right angles to the plane of section only refers to the cleavage. It would be of interest to test by the aid of vital staining marks whether in *Lytechinus* a cleavage axis perpendicular to the cut surface always coincides with the gastrulation axis. The same authors assume that the endoderm material occupies about 19/20 of the egg, but this assumption is in conflict with some of their own results (see H-s, 1937).

An isolated 1/2-blastomere or any meridional half of an early cleavage stage forms, during segmentation, a more or less open half blastula, which will soon close. In the closed blastula the most animal part lies close to the most vegetative (vital staining, H-s, 1928*b*, p. 62). The presumptive ectoderm and endoderm differentiate only as ectoderm and endoderm respectively, but the material within these germ layers will be used atypically, as the presumptive oesophagus may take part in the formation of the intestine, etc. (Fig. 3 B₂, B₃). Nevertheless, the animal-vegetative axis of the 1/2-larva will deviate only slightly from that of the egg (Fig. 3 B₁) (H-s, 1928*b*, p. 63, 1935, p. 280).

Many investigators have studied the development of more or less fused twins, obtained either by an incomplete separation of the first two blastomeres (or displacement of later blastomeres in relation to each other) or by the fusion of two

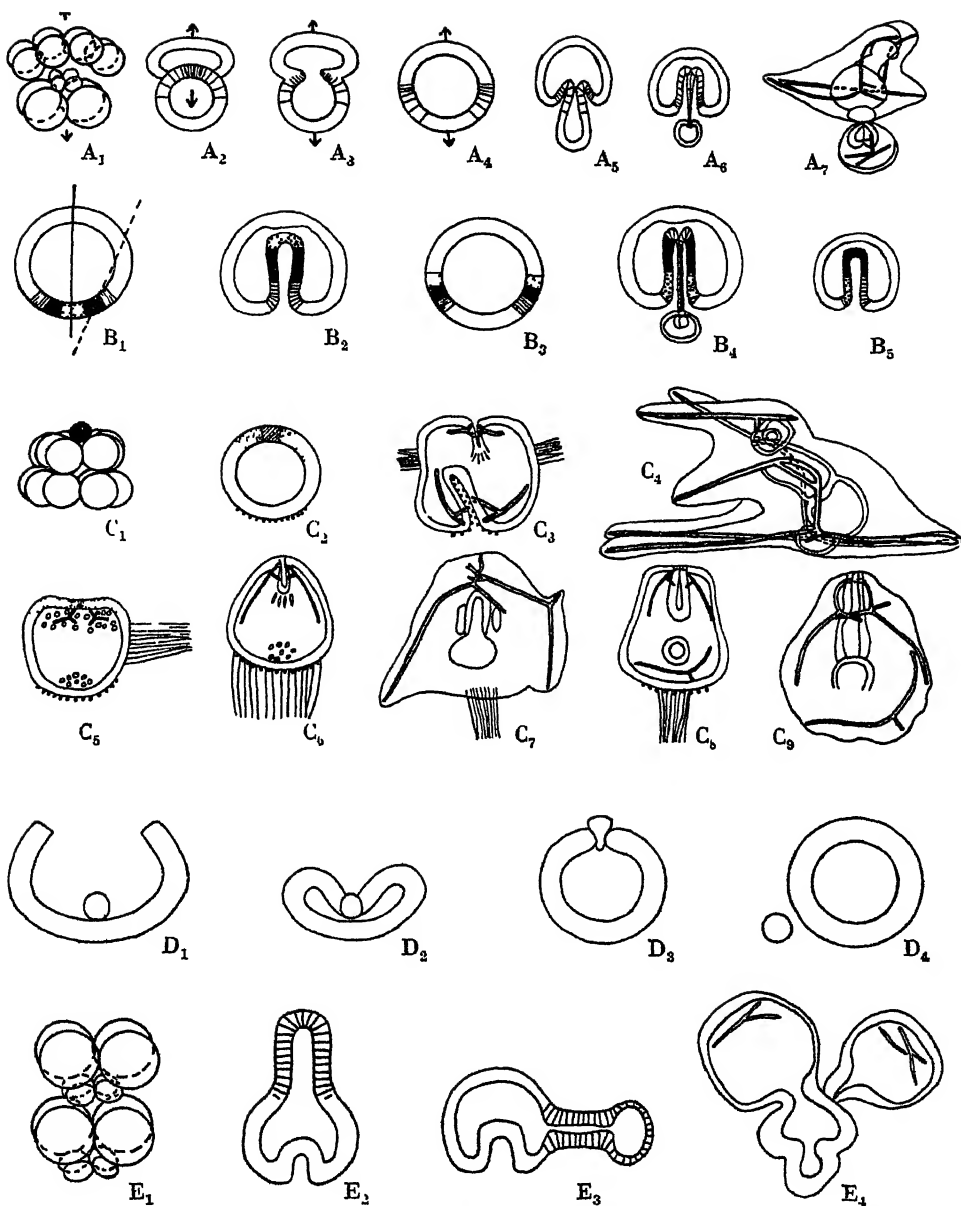


Fig. 3. A₁-A₇, diagram of inversion of the vegetative half. The polarity of the halves is indicated by arrows. The presumptive archenteron is stippled, the most vegetative material most densely stippled. Further explanation, p. 143. B₁, B₂, diagram of normal blastula and gastrula. Presumptive oesophagus dotted, stomach black, intestine stippled. Mesenchyme omitted. B₃, B₄, blastula and gastrula with reversed vegetative half (like A₁-A₇). B₅, the position of the material in a meridional half. The broken line in B₁ indicates the position of the new animal-vegetative axis in a half larva in relation to that of the entire egg. C₁-C₉, implantation of four micromeres (black in C₁, stippled in C₂) into the animal pole of an animal half. C₂, checking the site of implantation by use of two vital stains. C₃, C₄, larvae with two archenterons, one induced, animal, and one vegetative. C₅-C₉, reversal of the polarity. Further explanation, pp. 143 and 159. D₁, a piece of cytoplasm without nucleus put into an animal half before it has closed. D₂-D₄, the edges of the half blastula bend down, seize the cytoplasm and eject it (p. 22). E₁, a vegetative half of one 16-cell stage is placed on top of another. E₂-E₄, the upper half (stippled) begins to migrate along the side of the lower, until the most vegetative parts fuse. See p. 142. (H-s, 1928b, 1935.)

eggs, or cleavage stages, or blastulae. The methods employed for obtaining twins from one egg involve shaking, diluted sea water, change of the composition of the sea water, heat, cold, stretching, exovates, etc. Fusion has been brought about by packing eggs close together, with or without previous treatment with alkaline calcium-free sea water, etc. The results comprised all kinds of twins: double twins with two sets of organs but joined with the ectoderm, incomplete twins with organs partly doubled, partly unified, and harmonic larvae, in the case of fusion of two whole eggs with all organs of double size (Driesch, 1891, 1892, 1893*a*, 1896*c*, 1900*b*, 1902*b*, 1903*b*, 1906, 1910; Herbst, 1892; Loeb, 1894*a*, 1895; Morgan, 1895*a*; Boveri, 1901*b*; Garbowski, 1904; Janssens, 1904; Heffner, 1908; Bierens de Haan, 1913*a*, *b*; Bury, 1913; Goldfarb, 1914–17; Runnström, 1920*a*, 1925*b*; Newman, 1921*b*; v. Ubisch, 1925*b*; H-s, 1925*d*, 1928 *a*, *b*; Peter, 1931; Balinsky, 1932; Tyler, 1933, 1935). Boveri (1901*b*) assumed that the axes of the two components must point in the same direction for a single, harmonic larva to result. Bierens de Haan (1913*a*) added the condition that the axes must stand at the side of, not in line with each other. Von Ubisch (1925*b*), who stained the one component (as also Garbowski, Janssens, and Bierens de Haan did), stated that a unit developed when the two vegetative centres were lying close together. By transplantation, when the relative position of the two halves of a 16-cell stage was exactly known (H-s, 1928*b*, pp. 70–3, 80, 162), it was found that the axes need not necessarily be parallel: halves rotated 45° or 90° in relation to each other giving a whole larva, even if two invaginations occur to begin with, since the blastopores move towards each other and fuse. Thus, even if the tip of the archenteron has been forked, a single digestive tract may develop, although often the skeleton and the arms may be irregular, as the regulation of the ectoderm is not always complete. But if the halves are rotated through 135° or 180°, two separate archenterons always result.

A striking proof that two components placed with the axes in the same direction, but the one on top of the other, do not do well together is afforded by the transplantation of a vegetative half on top of another one, with the micromeres of the former against the most animal part of the latter (Fig. 3 *E*₁). The lower half begins to gastrulate. The upper half does not remain at the animal pole of the lower, but actually migrates along its side down to the most vegetative part of the lower partner. Here, the two presumptive endoderm areas together differentiate as endoderm, while the two presumptive ectoderm regions (*veg*₁) separately differentiate into ectoderm (Fig. 3 *E*₄). Thus the two endoderm areas try to join, but the large angle between the axes of the partners after the migration does not permit of the formation of a unit (H-s, 1928*b*, p. 79). On the other hand, in a few exceptional cases (Balinsky, 1932), a harmonic giant larva is obtained from two 4-cell stages, one on top of the other, with the axes directed similarly. In these cases we must assume a complete rearrangement within the two eggs into a single axis.

It was mentioned above that two meridional halves rotated through 90° gave, in the end, a uniform digestive tract, but the ectoderm and skeleton showed irregularities, relics of the old polarity. The ectodermal material may, however, also

completely change its polarity. If we add an animal half to a meridional, the axis of the former is perpendicular to that of the latter (Fig. 7 A_1). Nevertheless, in almost all cases we obtain a perfect pluteus (A_4). The vegetative material of the meridional half causes the adjacent presumptive ectoderm of the animal half to become endoderm (A_2, A_3), and by regulation a single axis is established for the new individual. It thus seems easier to obtain a complete regulation in a system with diverging axes when two animal centres, but only one vegetative centre, are present, than when to begin with the system possesses two vegetative centres (H-s, 1928*b*, pp. 70-8, 1935, pp. 348-50).

Small vegetative fragments (the four micromeres) also exert a remarkable influence upon polarity. If four micromeres are implanted in the side of an entire egg in the 32-cell stage, we find an archenteron, generally rather small, induced at the point of implantation and provided with a more or less complete supplementary skeleton (Fig. 7 B_1-B_3). Thus a second animal-vegetative axis has been induced (H-s, 1935, p. 392). The normal animal-vegetative axis does still exist, but if we take an isolated animal half—the normal vegetative centre thus being absent—and implant the micromeres at the animal pole of this animal half, the vegetative forces in the micromeres may in some cases bring about a complete reversal of polarity (Fig. 3 C_1, C_2, C_6-C_9 ; H-s, 1935, p. 405).

Another profound change is exhibited in the following experiment; but here it is the animal, not the vegetative material, that causes a reversal of the polarity in a part of the egg. If we cut between the animal and the vegetative half of a 16-cell stage and turn the vegetative half upside down (Fig. 3 A_1), the two parts will not form a blastula straight away, the reversed half turning inside out. On the contrary, each partner will round up in its own manner. We therefore obtain a small, vegetative blastula with the animal half perched like a cap on its top (Fig. 3 A_2). Then the small vegetative blastula opens into the cap, and we get just one round blastula, in which the most vegetative material is now located immediately below the equator (Fig. 3 A_3, A_4, B_3). Gastrulation starts with the most vegetative material (A_5). The result will be a pluteus with a sort of gastrula hanging outside the anus, the polarity of the gastrula being reversed to that of the pluteus (Fig. 3 A_6, A_7). The gastrula is derived from the presumptive ectoderm and a part of the presumptive endoderm of the vegetative half, which was reversed at the time of operation. But the endoderm of the pluteus also comes from the vegetative half. Thus the animal half has incorporated a part of the presumptive endoderm of the vegetative half, actually reversing the polarity of the incorporated part (Fig. 3 B_2, B_4). Lithium causes endoderm formation (Herbst, 1892, 1893, 1896). If larvae with reversed vegetative halves be put in lithium, it is found to act along the two vegetative axes separately, thus partly along the vegetative-animal axis of the pluteus, partly in the reverse direction along the vegetative-animal axis of the little gastrula (H-s, 1936*b*, p. 51).

When eggs were treated with NaSCN before fertilization, Lindahl (1933, 1936*a*) obtained an ectodermization of the whole egg, and in some cases not only an enlargement of the apical organ at the animal pole, but also a new apical organ

at the vegetative pole; thus there seems to be a reversal of polarity in the vegetative part of the egg. It is also interesting to note that Runnström (1925*b*, 1926), after treatment with diluted sea water, found small supplementary archenterons anywhere on the gastrula—a sort of “anarchy of the axis”.

The experiments mentioned above show that the polarity of the egg is fairly stable, as it is not altered by centrifuging or moderate stretching, and as it is more or less retained in fragments. On the other hand, the polarity can be changed both by a more considerable stretching, and if animal and vegetative material are placed in atypical relations to each other: a new axis may be induced, and the whole polarity may even be reversed. It must be borne in mind that a reversal can be brought about both by vegetative and animal material.

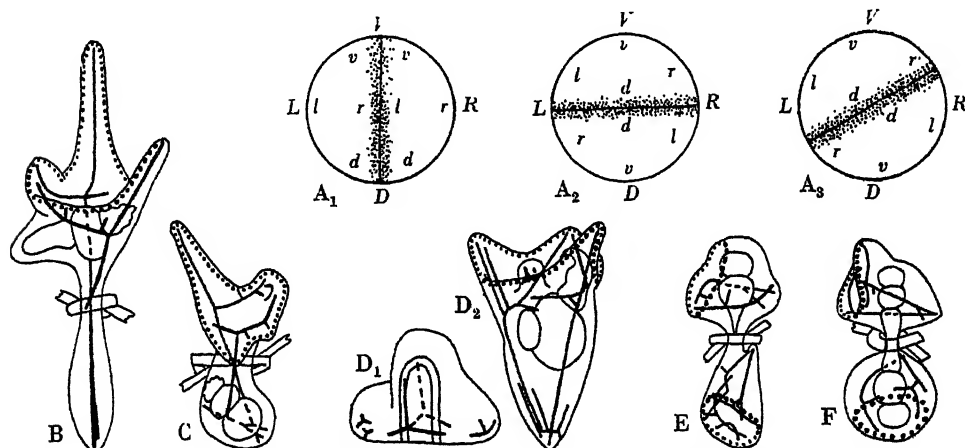


Fig. 4. A_1 – A_3 , diagram of the position of the dorso-ventral axis in right and left halves (A_1), ventral and dorsal halves (A_2), and in ventro- and dorso-lateral halves (A_3). V , ventral, D , dorsal, L , left, and R , right side of the entire egg; v , d , l , r the same of the halves. B – F , meridional constriction with a silk fibre at the 16-cell stage. B , C , only one dorso-ventral axis, in B with the archenteron in the ventral, in C in the dorsal part. D_1 , to begin with there are two dorso-ventral axes, the larger behind the smaller, thus no reversal. Later the small partner becomes incorporated in the larger (D_2). E , F , in cases of severe constrictions the dorso-ventral axis is inverted in the dorsal half. Cf. p. 146.

It is important to note that the inverted vegetative half in the experiment of Fig. 3 A does not take part in the formation of the blastula directly by turning inside out, but rounds up and closes in its normal direction (Fig. 3 A_2) (the same thing was found by Peter, 1931). This proves the existence of a radial polarity (H-s, 1928*b*, pp. 68, 99), as suggested already by Boveri (1901*b*) and Runnström (1925*d*). Child (1936 *a*, *b*, p. 484) found a reduction gradient from the blastocoele outwards. The experiments indicate that this radial polarity cannot be reversed.

The dorso-ventral axis is less stable than the animal-vegetative and radial ones, as its position is readily influenced by stretching: the median plane always coincides with the stretching axis, in so far as this axis is not identical with the egg axis (Boveri, 1901*b*; Lindahl, 1932*b*, 1936*a*; H-s, 1938 by constriction, Fig. 4 B , C). In some cases the stretching or constriction may lead to a reversal of the dorso-ventral axis in a part of the egg (see below). Also the centrifugal force affects the

position of the dorso-ventral axis (Runnström, 1925*c*; Lindahl, 1932*c*, 1936*a*). By the use of chemicals, such as lithium, sodium butyrate, sulphate free sea water, etc. (Herbst, 1897, 1904, and others) the bilateral symmetry may be deranged or eliminated, so that larvae with a radial symmetry appear. The stronger the action of lithium is, the more the dorso-ventral axis is rotated, its ventral pole moving towards the animal pole, its dorsal towards the vegetative (Herbst, 1893; Runnström, 1928*b*, 1929, 1931). The same translocation takes place in vegetative fragments (Runnström, 1928*a*, 1929; H-s, 1935).

It is essential to know the position of the first furrow in relation to the plane of symmetry of the larva. Opinions vary on this. While Boveri (1901*b*, 1902, 1905, 1907), Garbowski (1905*b*), Herbst (1907), Jenkinson (1911*b*), and Runnström (1914) thought the first furrow coincided with the plane of symmetry, Driesch (1906, 1908*a*) considered the first cleavage plane to be frontal, but his observations refer to slightly stretched eggs (cf. the paragraph above). Von Ubisch (1925*a*) used local vital staining to solve the problem, and he came to the conclusion that the first furrow has no particular relation to the median plane of the egg. Runnström (1925*c*), Hörstadius (1928*b*), and Foerster & Örström (1933) thought (after vital staining) that the first furrow was more often formed in the median and the frontal plane than obliquely to those planes. Hörstadius & Wolsky (1936), however, found only a slight, if any, preponderance of the median and frontal planes. The essential point is that 1/2-larvae, isolated in the plane of the first furrow, correspond to dorsal or ventral, right or left, or oblique meridional parts of the egg.

Jenkinson (1911*b*) suggested that the dorso-ventral axis in the sea urchin's egg is induced by the sperm. Hörstadius (1928*b*, p. 21) stained the side opposite the point of entry of the sperm and found this point in all positions in relation to the first furrow (see above, p. 137). As the latter was considered to have either a median or a frontal position, Hörstadius drew the conclusion that the sperm does not determine the bilateral symmetry. But as the position of the first furrow seems to be more variable than was believed at that time (cf. the paragraph above) the grounds for the conclusion have changed, and the problem calls for reinvestigation. However, complementary deficiencies in meridional halves from unfertilized eggs (below, p. 168) indicate that a bilateral organization is already established in the egg before the entrance of the sperm (H-s & Wolsky, 1936).

Many different opinions have been advanced regarding the determination of the dorso-ventral axis in half-larvae. Boveri (in Heffner, 1908) assumed that it was rotated through 90° relative to that of the intact egg. Driesch (1906, 1908*a*) studied fused twins and came to the conclusion that the axis is kept in a dorsal partner, but reversed in the ventral. Runnström (1914) assumed the axis to remain unchanged in halves, judged as right and left halves. Vital staining of the cut side (or the side opposite to the cut side) of halves kept in pairs gave the following results (H-s & Wolsky, 1936). In right and left halves, as well as in ventral halves, the dorso-ventral axis does not change its position, but in dorsal halves it is reversed, the new ventral side thus being formed at the presumptive dorsal side (Fig. 4 A_1 , A_2). Also in the more dorsal dorso-lateral halves the axis is inverted (Fig. 4 A_3).

In more lateral dorso-lateral halves and in ventro-lateral halves it is not reversed but probably sometimes slightly rotated.

When Lindahl (1932*b*) stretched eggs by sucking them up into a fine pipette and stained the posterior end, he found that the stained side became ventral, provided the staining was moderate. With too intense staining it became dorsal. Lindahl assumes differences in metabolism at the anterior and posterior end of the eggs stretched in the pipette. Injurious staining would reverse the relation between the two ends. In some cases Lindahl (1932*b*, 1936*a*) obtained larvae with two ventral sides, thus both ends seem to have been equal. This implies a reversal of the dorso-ventral axis in some of the eggs in relation to the remainder, as in the separated dorsal halves. Lindahl assumes on the ventral side a centre which normally checks the formation of a similar centre on the opposite side. Lindahl's observations on stretched eggs were confirmed by constriction experiments (H-s, 1938). With slight, meridional constrictions we get only one ventral side, and it develops irrespective of the position of the archenteron (which may lie in the ventral, or the dorsal part, Fig. 4 B, C). When the eggs are more strongly constricted, the dorso-ventral axis may be reversed in the dorsal partner (Fig. 4 E, F). If the dorsal part of a slightly constricted egg at an early stage becomes larger than the ventral, the dorso-ventral axis may also be divided into two, but without reversal, as the one axis will lie behind the other (Fig. 4 D₁). In this case the egg-axis has not been rotated (H-s, 1938). The smaller partner will later become incorporated by the larger (Fig. 4 D₂).

The faculty of inverting the dorso-ventral axis in a dorsal half ceases in the late blastula, about at the beginning of the mesenchyme formation (H-s, 1936*a*).

We have seen examples of reversal of polarity of the animal-vegetative axis, but only as a consequence of transplantations (or chemical action): reversal of the vegetative half (Fig. 3 A) or implantation of micromeres in the animal pole of animal halves (Fig. 3 C). The less stable dorso-ventral axis adjusts itself in conformity with the stretching axis, and with considerable stretching or constriction, which involves partial physiological isolation, as well as at complete isolation, the axis is spontaneously reversed in the dorsal half.

If an archenteron is in an irregular position in relation to the symmetry of the ectoderm, it will adjust itself to the latter (Runnström, 1925*b*; H-s & Wolsky, 1936).

Echinoderm larvae have a marked bilateral asymmetry, the hydrocoele (which gives rise to the ambulacral system) and the hydropore developing on the left side. A few cases of larvae with reversed asymmetry, or with hydrocoele on both sides, have occasionally been observed among sea urchins, brittle stars, starfishes, and holothurians (lit. see H-s, 1928*b*, p. 163; Schleip, 1929, p. 512), or found after treatment with hypertonic sea water (MacBride, 1918, *Psammechinus*), or after being reared in too little water (Runnström, 1918, *Paracentrotus*), or at low temperature (Newman, 1921-5, *Patiria*). Runnström (1918) assumes the presence of three axes already in the uncleaved egg, each with the opposite poles different. The determination of the right-left axis has not been much studied in sea urchins.

We have more information as regards starfishes. The preponderance of the left side is generally rather marked. In *Psammechinus* larvae with twisted archenteron only one coelom was budded off, dorsal or ventral; in the majority of cases the coelom grew a hydropore on the left side (Runnström, 1925*b*). Left halves of starfish blastulae or gastrulae always developed the hydrocoele and the hydropore on the left side; in right halves on the left, or the right, or on both sides (*Henricia*, *Solaster*, Runnström, 1920*a*; *Asterina*, *Astropecten*, H-s, 1925*a*, *b*, 1928*b*). In *Holothuria* only the left halves were able to develop (H-s, 1925*c*, 1928*b*). The results in the case of the starfish were explained on the basis of two opposite, overlapping gradients, the left stronger than the right. It then appears to depend upon the relative strength of the gradients whether in the right halves the left or the right will be the stronger, or whether they will be equal (H-s, 1925*c*, 1928*b*). Runnström (1920*a*) fused two left halves of gastrulae of *Henricia*, and also two right halves. In the former case the larva developed two ambulacral, in the latter two antiambulacral sides. The determination as regards the characters right-left was already accomplished. In partially fused twins the asymmetry of the partners was independent of each other (*Psammechinus*, Runnström, 1925*b*).

VI. DETERMINATION ALONG THE EGG AXIS

Boveri (1901*b*) spoke of a stratification along the animal-vegetative axis in the sea urchin's egg, with layers of different physiological qualities, and he also used the word gradient (*Gefälle*, 1910*a*). The most vegetative part would exert a special, determining influence upon the rest of the egg. If the most vegetative material be removed, the material next to it would act as its substitute, provided it was still so vegetative that it could do so. Morgan (1905) considered polarity as a phenomenon of gradation of materials. Child (1907) defined polarity as axial physiological differentiation. Heffner (1908) and Runnström (1914) assumed gradients of concentration. Child (1915, 1916*a*, *b*, 1928, 1929, 1936*a*, *b*) and his school showed the existence of layers of different physiological susceptibility in the eggs of starfishes, sea urchins, and other groups (axial gradients). As a result of susceptibility experiments, Runnström (1928*a*, *b*, 1929, 1931, 1933) suggested that there exist along the egg axis in the sea urchin not only one, but two gradients, one animal and one vegetative, which both reach to the opposite pole, thus overlapping through the whole egg, and which interact mutually and are partially hostile to each other. This implies two gradients of qualitatively different nature. On the other hand, Child (1936*a*, *b*) maintains that there is only one kind of gradient, which is illustrated by an animal-vegetative gradient of reduction in the cleavage and early blastula stages. The phenomena which support the view of an opposing vegetative-animal gradient are explained by Child on the basis of the fact that at the time of primary mesenchyme formation and gastrulation there appears a new reduction gradient in the vegetative part. This new gradient is reversed in relation to the first one, which still persists in the animal part. The investigations described below show—to judge from the differentiation of larvae of atypical constitution—that in the sea urchin's egg we have to deal with animal and vegetative qualities which

must interact to bring about typical differentiations, and also that the one factor may suppress the other. The experiments illustrate furthermore how the animal qualities decrease from the animal, the vegetative from the vegetative pole. It seems thus as if we have to deal with two different gradients.

While Driesch (1891, 1892, 1893*a*, 1896*d*, 1898*a*, 1899*a*) considered all parts of the sea urchin's egg to be totipotent, the egg being a "harmonic equipotential system", Zoja (1895), Boveri (1901*b*, 1902), Terni (1914), v. Ubisch (1925 *c*, *d*, 1929, 1932*a*), Hörstadius (1928*b*, 1931, 1935 1936*a*), and Plough (1929) have found that animal halves do not gastrulate, nor form any skeleton. Generally their apical tuft is much enlarged (Zoja, 1895; Driesch, 1900*a*; Terni, 1914; Runnström, 1928*a*; H-s, 1928*b*, 1931, 1935). The result found by Driesch (1900*a*, 1902*b*) that at least about 25 % of the animal halves develop into plutei has been explained by Hörstadius (1928*b*, p. 124) as an error. Driesch shook 8-cell stages and isolated groups of four cells. Those which did not form any micromeres on subsequent cleavage were considered as animal fragments. But as the micromere formation is often inhibited after shaking, Driesch must in many cases have isolated meridional and vegetative fragments as animal ones, and these account for the gastrulae and plutei obtained. Von Ubisch (1933*c*) reports three gastrulating animal halves of *Echinocyamus*, but these cases do not count, as v. Ubisch expressly states that the females were overripe (cf. H-s, 1935, p. 435, 1938, p. 229).

E. B. Harvey (1932, 1933) centrifuged unfertilized sea urchin's eggs in a sugar solution, until they were separated into half-eggs. These were of course different as regards the strata they contained (yolk, oil, granules, pigment, etc.). With greater centrifugal force, the colourless half-eggs could be separated into quarter-eggs. All the fragments could be fertilized and cleaved, those without an egg nucleus being haploid. Some fragments developed into dwarf plutei. As the axis of stretching and plane of separation evidently form any angle to the egg axis, the fragments must be very varied as regards their animal and vegetative properties.

If we study the isolated animal halves closely (H-s, 1935, pp. 283-315), we find that the majority shows a greatly enlarged apical tuft, covering up to about three-fourths of the surface of the blastula (Fig. 5 A₁). Others have a smaller apical tuft, and there are all kinds of intermediate stages, down to some animal halves with a normal apical tuft (Fig. 5 A₂-A₄). A couple of days later those with a large apical tuft develop into uniformly ciliated blastulae (Fig. 5 A₅). Those with a slightly smaller apical tuft may differentiate in the same way, but they generally produce blastulae with a thin pavement epithelium on one side and a ciliated field of the same type as the wall of the ciliated blastula on the other (Fig. 5 A₆). Others form blastulae with a ciliated band (Fig. 5 A₇), and those with a normal or only slightly enlarged apical tuft generally develop into blastulae with a ciliated band and a stomodaeum, which is thus formed without any trace of an archenteron being present (Fig. 5 A₈). Such larvae had been observed by v. Ubisch (1932*a*, 1933*c*).

The vegetative halves also exhibit different types (Fig. 5 B₁-B₈) (Zoja, 1895; Driesch, 1905*b*; H-s, 1928*b*, 1931, 1935, 1936*a*; Runnström, 1928*a*, p. 573; v. Ubisch, 1929). Many of them have no apical organ, and most of them are some-

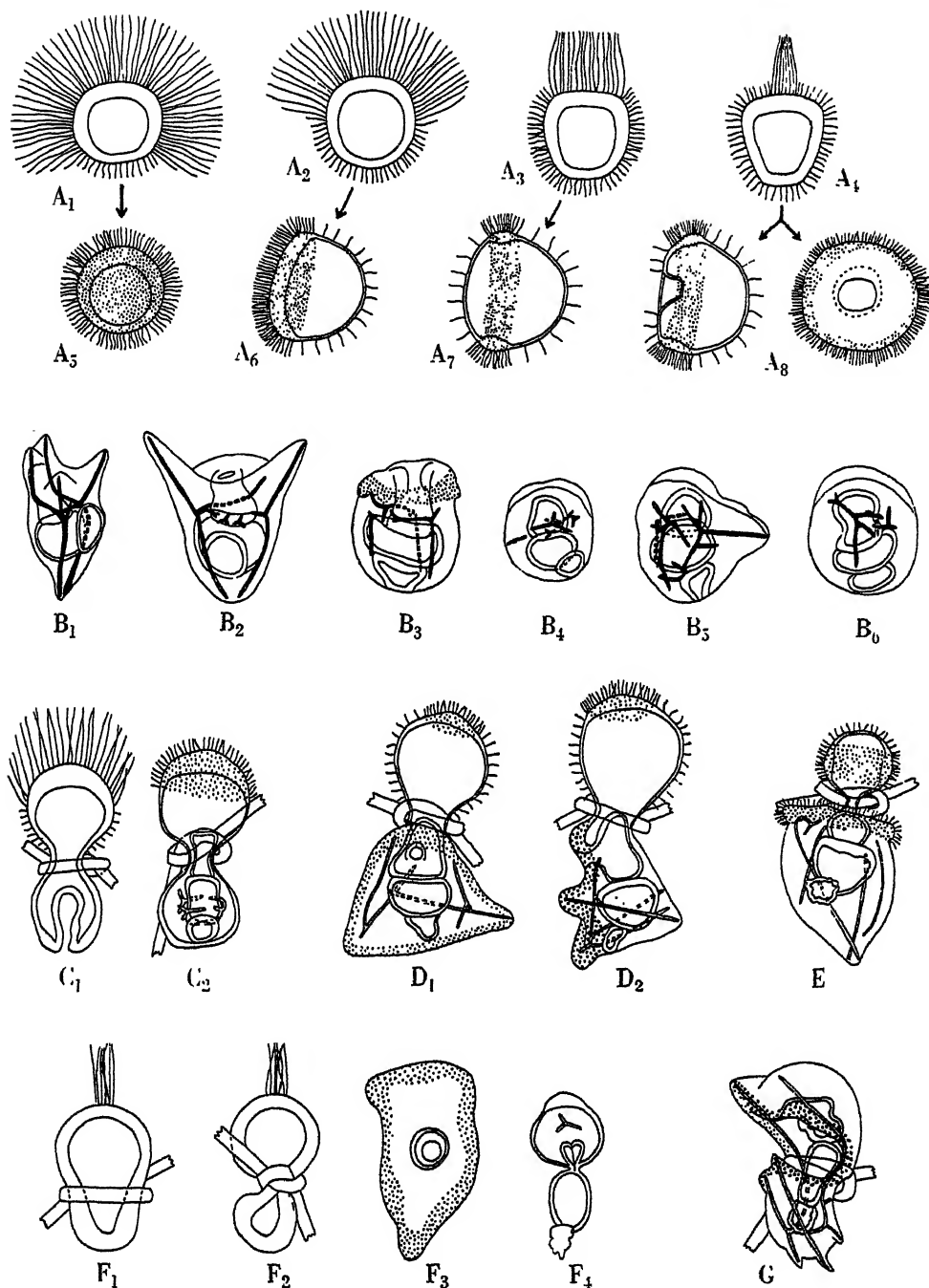


Fig. 5. A₁-A₈, development of isolated animal halves. A₁-A₄, young blastulae with more or less enlarged apical tuft. A₅-A₈, fully differentiated halves. B₁-B₆, development of isolated vegetative halves. The animal and vegetative halves on the left represent equatorial eggs, those to the right subequatorial eggs (see pp. 148-51). C-E, equatorial constriction in early cleavage stage. F-G, the originally loose hair-loop has at a late blastula stage been more tightly constricted. (Further explanation to C-G, p. 163.) (H-s, 1935, 1938.)

what ovoid in shape, without arms, with a poor and irregular skeleton, and often without mouth. Others may have an apical organ, mouth, etc., and more or less resemble a pluteus. A few look just like small plutei (Fig. 5 B₁-B₆). There is a great resemblance between vegetative fragments and lithium larvae (cf. p. 135).

As mentioned above, Driesch considered the sea urchin's egg to be a harmonic equipotential system: practically every fragment would develop into a whole larva. But we now find that the animal half, which normally gives rise to the stomodaeum and a part of the ciliated band, after isolation in most cases does not even form these organs but only differentiates into a ciliated blastula or a blastula with a ciliated field. In the vegetative halves too we find a development that does not lead to the more harmonic differentiation that Driesch's conception would require, but to an even less harmonic larva than the prospective significance of the material would indicate. A vegetative half contains nearly a third of the presumptive ectoderm (*veg*₁) and the entire presumptive endoderm and mesenchyme. For the emergence of a harmonic larva an ectodermization of presumptive endoderm ought to take place. But, on the contrary, the quantitatively dominating endoderm material suppresses a part of the presumptive ectoderm, which, at least in many cases, becomes endodermized, so that the archenteron in the vegetative half is larger than in the normal larva (H-s, 1936*b*). It is possible that no such translocation of the ecto-endoderm border occurs in those vegetative halves that differentiate into typical plutei, but that point has not been investigated. Although the archenteron is enlarged, the primary mesenchyme cells do not increase in number (H-s, 1936*b*).

It is possible to understand the differentiation of the vegetative halves if we assume that, after isolation, a rearrangement of the gradient system takes place, bringing about a stronger concentration of the animal and vegetative properties at the poles than in the same material before fragmentation. In that way the gradients will become steeper, the system will resemble a normal one more closely than it did when still in connexion with the animal half. Thus we find both more animal differentiations than the prospective significance of the material (apical tuft, mouth, oral arms, etc., in some of the vegetative halves), and more vegetative (the enlarged archenteron) (H-s, 1935, pp. 371, 457, 474, 1936*b*). In other halves the animal gradient was not strong enough to cause, after the rearrangement, the formation of such organs as apical tuft, mouth, etc. (ovoid larvae).

In most animal halves the vegetative properties are so to say covered by the animal ones: the apical tuft is extended and neither ciliated band nor stomodaeum are formed (Fig. 5 A₁, A₂, A₅, A₆). In other cases the vegetative properties in the animal half are capable of inhibiting the extension of the apical tuft and of causing the formation of both ciliated band and stomodaeum (Fig. 5 A₄, A₈). These differences in the development of animal and vegetative halves are to be explained as follows. If we rear the animal and the vegetative half from one egg together, we find that, roughly speaking, the animal halves with enlarged apical tuft correspond to the more harmonically differentiated vegetative halves, whereas the more richly differentiated animal halves correspond to the ovoid larvae (e.g. Fig. 5 A₁, A₅, B₁ and A₄, A₈, B₆, respectively). It was mentioned on p. 133 that the third, equatorial

furrow is often translocated towards the vegetative pole (subequatorial eggs). The animal halves with stomodaeum and ciliated band come from subequatorial eggs. The additional material which, owing to the position of the third furrow, they have gained from the vegetative half, inhibits the extension of the apical tuft and causes the formation of the stomodaeum and ciliated band. On the other hand, the lack of the most animal part of the subequatorial vegetative half accounts for the poor development of the ectoderm and skeleton (which depends upon the ectoderm, see p. 135) of the vegetative half. It is not always the position of the third furrow that determines the type of differentiation, as the gradients may overlap in different ways (H-s, 1935, p. 309).

By the following experiment we can prove the correctness of the view that the formation of a typical apical tuft and of a ciliated band and a stomodaeum is not self-differentiation but is due to influences from material below the equator. If we take eggs of the equatorial type (animal halves that give enlarged apical tuft, later ciliated blastulae, Fig. 6 A; the eggs from one female are often of the same type) and isolate the entire presumptive ectoderm ($an_1 + an_2 + veg_1$), we find that this fragment develops into a blastula with a normal apical tuft, a ciliated band and a stomodaeum ($8 + veg_1 + 0$, Fig. 6 B) (H-s, 1931, 1935; v. Ubisch, 1933 *a, c*). Thus the veg_1 material has inhibited the extension of the apical half and together with the animal half has brought about the differentiation of ciliated band and stomodaeum. In subequatorial eggs, as a consequence of the translocation of the third furrow, the furrow veg_1-veg_2 also apparently lies farther towards the vegetative pole than in equatorial eggs, as $8 + veg_1 + 0$ of subequatorial eggs form a small archenteron, and often also some skeleton. Thus in those cases the fragment contains a small part of the presumptive endoderm (H-s, 1931, 1935, p. 323; v. Ubisch, 1933 *c*, p. 63). The appearance of the archenteron, and particularly of the skeleton, may be due in part to reconcentration of the kind described above.

If veg_2 instead of veg_1 is added to an animal half, we obtain a perfect pluteus (Fig. 6 D). In this case the archenteron is formed from veg_2 , in conformity with its prospective significance. Moreover, veg_2 has the faculty of checking the apical tuft and bringing about the differentiation of the ciliated band and stomodaeum; by regulation it also gives rise to skeleton (H-s, 1931, 1935, p. 324). Driesch (1893, 1905 *b*, 1908 *b*) had already studied fragments without micromeres, but the results are not clear. It has since been found that whole eggs with micromeres removed can produce a skeleton (H-s, 1928 *b*, p. 80, 1935, p. 317, 1938, p. 231; Plough, 1929; v. Ubisch, 1931 *b*, p. 195; and in Schmidt, 1936, p. 235). Thus the most vegetative type of cells, namely, skeletal cells, can appear after removal not only of the micromeres, but, in some cases, also of veg_2 . In the same way the most animal differentiation, the apical tuft, is formed, and a fine pluteus is developed, after removal of the most animal quarter of the egg (v. Ubisch, 1929; H-s, 1935, p. 315), and, in some cases, also after removal of the entire animal half (see above). These facts again illustrate the reconcentration at the poles.

The following is a very strange phenomenon. If $8 + veg_1 + 0$ (which, when isolated, gives typical apical tuft, ciliated band and stomodaeum (Fig. 6 B),

possibly, in subequatorial eggs, also archenteron and skeleton) is divided into four meridional parts (one shown, Fig. 6 C₁) these first have a small apical tuft, as one

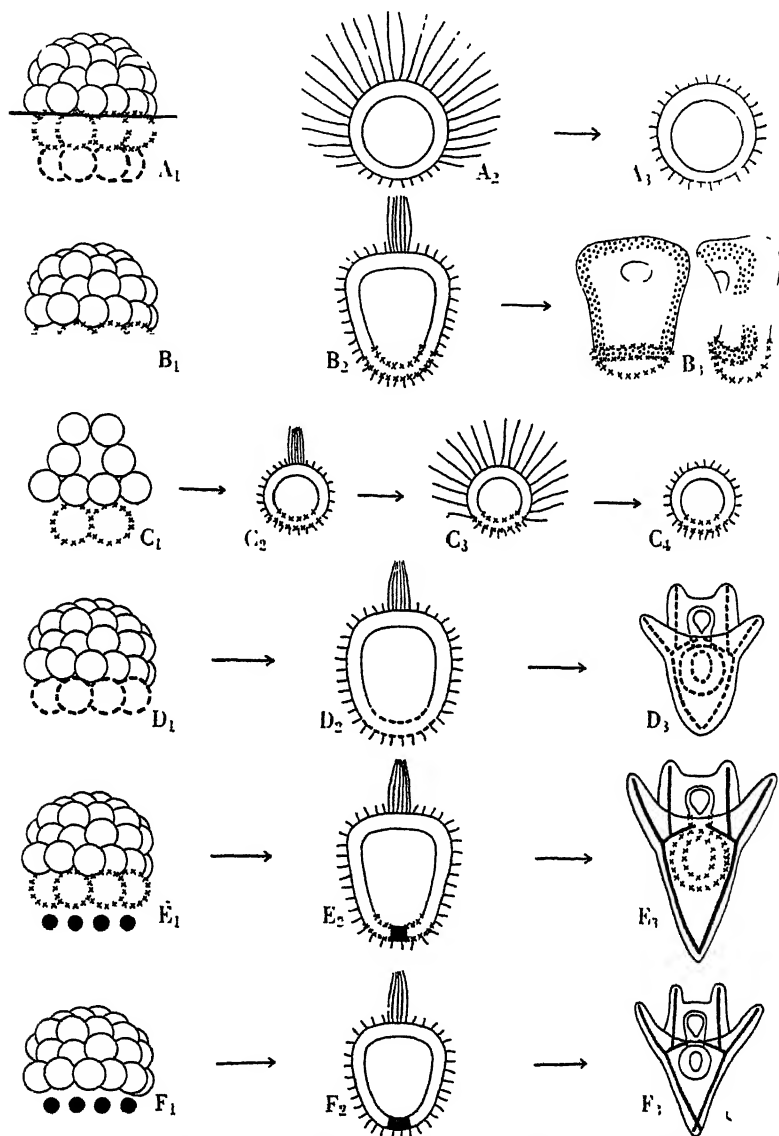


Fig. 6. Diagram of the influence of *veg*₁, *veg*₂, and the micromeres on the differentiation of equatorial animal material. *an*₁ and *an*₂, continuous lines; *veg*₁, crosses; *veg*₂, broken line; micromeres, black. A, isolated animal half. B, 8 + *veg*₁ + 0. C, a meridional quarter of 8 + *veg*₁ + 0. D, 8 + *veg*₂ + 0. E, 8 + *veg*₁ + 4. F, 8 + 0 + 4. (Further explanation, pp. 151-3.) (H-s, 1935, 1936b.)

would expect (Fig. 6 C₂), but soon the tuft becomes very much enlarged (C₃), and the fragments develop only into ciliated blastulae, like animal halves (C₄). This is not because the fragments are too small to differentiate, as four more vegetative

fragments of the same size ($\frac{an_2+4+0}{4}$) gave plutei. Nor is it due to qualitative differences between the dorsal and ventral, or right and left parts of the egg, for both dorsal and ventral, as well as right and left halves of $8+veg_1+0$ develop into larvae with ciliated band and stomodaeum. Thus the vegetative forces, when quantitatively below a certain level, cannot resist the animal ones, although their relative amounts are the same as in the entire $8+veg_1+0$ (H-s & Wolsky, 1936). So far we know of no explanation of this phenomenon.

Implantation of micromeres into animal fragments of early cleavage stages causes a profound change of differentiation (H-s, 1931, 1935). If implanted in the entire presumptive ectoderm ($8+veg_1+4$) the result will be a perfect pluteus, hardly to be distinguished from a normal one (Fig. 6 E). The micromeres themselves produce the skeleton and have induced the archenteron, which is thus formed from presumptive ectoderm. The same thing happens if we implant the micromeres in an animal half (Fig. 6 F) or in an an_1 ring (Fig. 9, an_1+0+4). The micromeres thus have the power to convert also an_2 and even an_1 material to endoderm. Moreover, they inhibit the extension of the apical tuft and cause the formation of ciliated band and stomodaeum (H-s, 1931, 1935, 1936b). If, however, only one or two micromeres are implanted, they often do not manage to check the apical tuft, and only in a few cases are they strong enough to induce an archenteron (Fig. 9).

The experiments illustrated in Fig. 6 thus show that the animal half of equatorial eggs develops into a blastula with greatly enlarged apical tuft, and, later, without ciliated band and stomodaeum. The entire vegetative material (veg_1 , veg_2 , the micromeres) has the faculty of inhibiting the extension of the apical tuft and bringing about the differentiation of the ciliated band and stomodaeum. Thus these potencies seem to begin at the equator. At the level veg_1-veg_2 we find the upper limit of the endoderm-forming material, a little farther down that of the skeletogenous material (H-s, 1935, p. 324; see also above, p. 151). The material below the presumptive endoderm (the micromeres) can induce endoderm. We thus get an illustration of increasing vegetative properties towards the vegetative pole.

Isolated animal halves in lithium may develop into plutei (v. Ubisch, 1925c, 1929). This does not show, as v. Ubisch maintains, that determination in the animal half is independent of the vegetative half, but only that the lithium has brought about the same kind of endodermization, etc., as four implanted micromeres ($8+0+4$, Fig. 6 F.) By implanting the most vegetative part of an animal half treated with lithium into another animal half Hörstadius (1936b, Fig. 12) was able to show that this material, which corresponds to a part of an_2 , now has the same power as micromeres.

It has been tested whether pieces of the most vegetative cytoplasm (without nucleus) of a fertilized egg will act in the same way as micromeres, when implanted in an animal half, but no such effect was found. The experiment revealed two extraordinary properties of the blastomeres in late cleavage stages: they are able not only to distinguish a piece of fresh cytoplasm without nucleus from a complete cell, but also to eject the former (Fig. 3 D₁-D₄) (H-s, 1935, p. 358).

The endodermizing power of the most vegetative material can be demonstrated in many ways; for instance, by adding an animal half to a meridional half (Fig. 7 A_1). A larva so produced has the volume of a normal larva but only half the vegetative material of a normal egg. By vitally staining the animal half, we can follow the endodermization which produces a perfectly typical pluteus (Fig. 7 A_2 - A_4) (H-s,

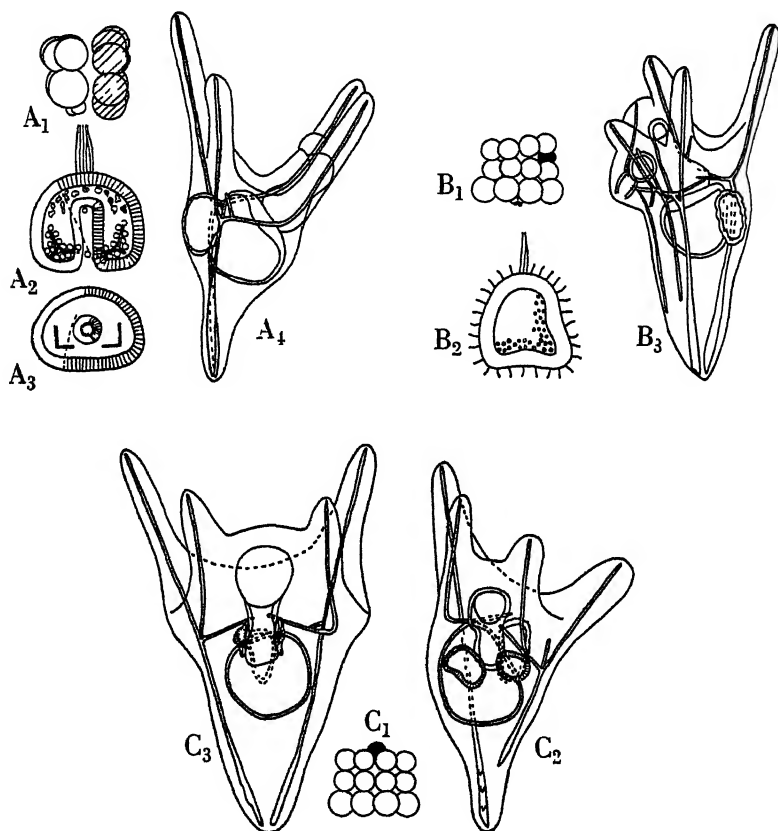


Fig. 7. A_1 - A_4 , fusion of a meridional half with an animal half (stippled) (see pp. 143, 154). B_1 - B_3 , implantation of four micromeres between anu_1 and anu_2 of a 32-cell stage (see pp. 143, 158). C_1 , the micromeres moved from the vegetative to the animal pole of the same egg. As compared with the control larva (C_3), the digestive tract and particularly the coeloms were enlarged at the expense of the ectoderm (C_2) (see p. 159). (H-s, 1935.)

1928*b*, p. 76, 1935). The same phenomenon occurs if we remove two macro- and two micromeres from a 16-cell stage, thus diminishing the vegetative material relatively to the animal (Fig. 8 A) (H-s, 1931, 1935, p. 336), and also if an animal half is added to an entire egg (H-s, 1928*b*, p. 77, 1935, p. 350).

If we gradually diminish the vegetative material still more (Fig. 8 A-E), thus leaving animal halves in contact with two macro- and two micromeres ($8+2+2$), or two macromeres without micromeres ($8+2+0$), or one macro- and one micromere ($8+1+1$), or one macromere ($8+1+0$), or only half a macromere ($8+1/2+0$),

we find endodermization in the first four cases, stronger when the micromeres are present, but only proportionate to the vegetative material (H-s, 1935, 1936b). The smaller the amount of vegetative material, the smaller also the amount of endodermization. The micromeres evidently have a stronger capacity to bring

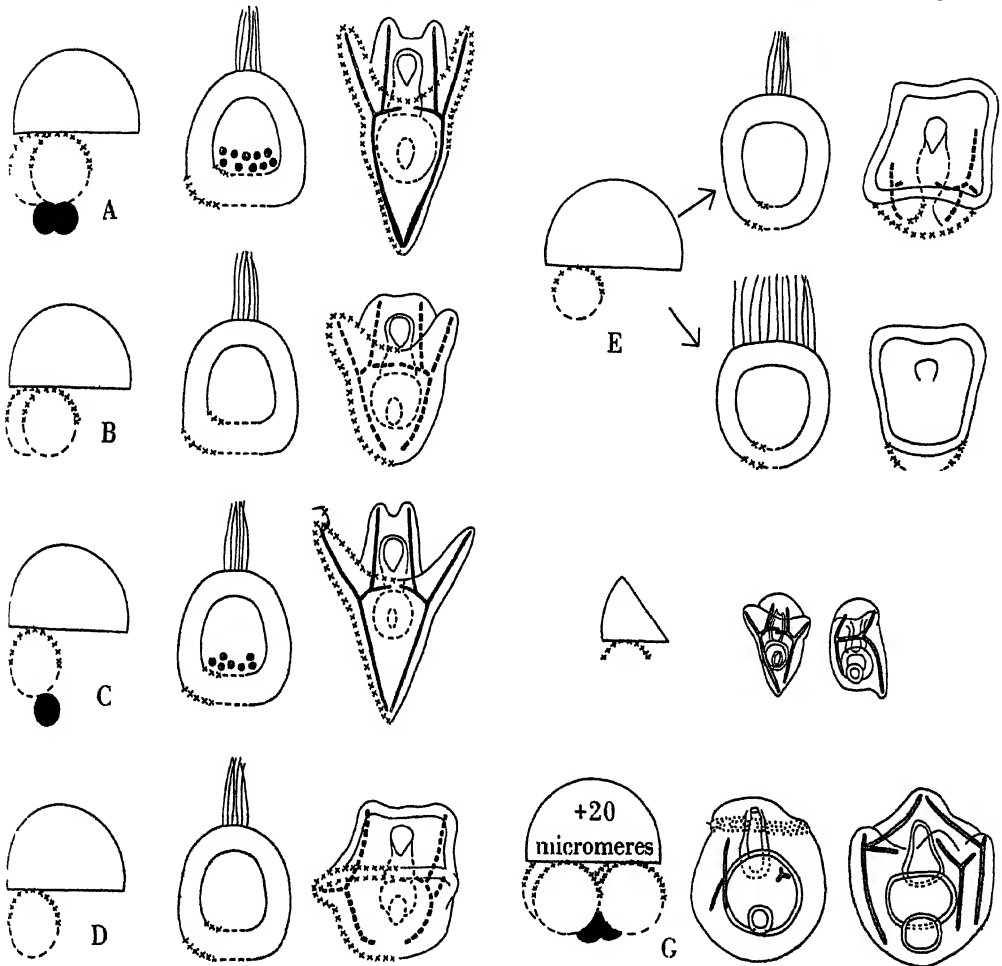


Fig. 8. Diagram of gradual diminution of vegetative material (A-E). Animal half=half circle, veg_1 , crosses; veg_2 , broken line; micromeres, black. A, $8+2+2$. B, $8+2+0$. C, $8+1+1$. D, $8+1+0$. E, $8+1/2+0$. F, if the animal material too is diminished, this small larva will differentiate more harmonically, as the animal and vegetative qualities are more typically balanced. G, if a whole egg be filled with micromeres, it will differentiate like a vegetative half (see p. 156). (H-s, 1935.)

about a typical differentiation than the same volume of macromere material (H-s, 1935, pp. 342-6). The endodermization in a larva $8+2+2$ points towards a regulation in the sense of Driesch, but the fact that the smaller the amount of vegetative material the smaller also the endodermization, is in conflict with Driesch, and if we diminish the vegetative material to a half macromere only ($8+1/2+0$) we

sometimes find that the presumptive endoderm of the half macromere cannot express itself: it is suppressed and ectodermized (Fig. 8 E, the lower larva).

That the differentiation of a harmonic larva out of a fragment is due, not to the absolute amount of animal and vegetative material present, but to the relative amount, is demonstrated by the following experiment. If we assume that in the last-mentioned experiment ($8 + 1/2 + 0$) the suppression of the presumptive endoderm has been caused by the animal forces, we ought to get a more normal development if we also diminish these animal forces, thus effecting a more typical equilibrium: that is to say, leaving only a few animal cells (instead of the animal half) in connexion with the half macromere. As a matter of fact such small fragments have a rather large digestive tract, and in most cases also a skeleton (Fig. 8 F). Thus the presumptive endoderm is no longer suppressed, and by regulation it may form a skeleton (H-s, 1935, p. 341). The same principle is exemplified also by the fact that we can turn animal halves or entire eggs into larvae of an ovoid, vegetative type by filling them with micromeres, which thus makes the gradient system resemble that of a vegetative half (Fig. 8 G) (H-s, 1935, pp. 352-5). (Only doubling the micromeres in a whole egg gives a typical pluteus with normal skeleton (H-s, 1928 b, p. 80; v. Ubisch, 1931 b, p. 226).) Furthermore, if the micromeres are removed from a vegetative half, we get a more harmonic differentiation than in a corresponding vegetative half with the micromeres present ($0 + 4 + 0$ and $0 + 4 + 4$ respectively; H-s, 1935, p. 355). This principle, that the relative, not the absolute, amounts are essential for differentiation, permits of our getting two plutei out of one egg after transverse section. It is not possible with one cut, as the vegetative fragment will exogastrulate, if the animal is so large as to form a complete digestive tract and skeleton. But if we cut twice and fuse the most animal with the most vegetative layer, we obtain two plutei: $8 + 0 + 4$ and $0 + 4 + 0$, or $an_1 + 0 + 4$ and $an_2 + 4 + 0$ (H-s, 1936 b).

The gradients can be clearly demonstrated by several other experiments. Let us first isolate the five layers spoken of before (see p. 133) (Fig. 9). The whole surface of an_1 is covered with long, stiff cilia. Later on an_1 differentiates into a uniformly ciliated blastula (Fig. 9 an_1). In an_2 the apical tuft covers about three-quarters of the surface. an_2 thus behaves more like an animal half, while an_1 is more animal than an animal half. veg_1 of subequatorial eggs may result in a fairly good archenteron and a skeleton. The equatorial veg_1 may show two types of differentiation. In both cases there is at first no apical tuft. In the more animal type the blastula tardily acquires an apical tuft and then develops into a ciliated blastula. In other cases no stiff cilia are formed at all, but a very much delayed, small archenteron appears (Fig. 9). Thus in the equatorial veg_1 layers we are in a region where, after rearrangement of the forces, either the animal or the vegetative may dominate. veg_2 illustrates very nicely the principle that the reorganization of the gradient system in fragments leads to a stronger concentration of the animal and vegetative forces at the poles of the fragment. It gives us ovoid larvae with a large bi- or tripartite archenteron and one or two small spicules (Fig. 9 veg_2). No apical tuft is formed. Here for the first time we encounter in a vegetative fragment ectoderm,

formed by regulation from presumptive endoderm. This means a more animal differentiation than is suggested by the prospective significance of the material. At the same time the skeleton is a more vegetative differentiation. Another interesting feature of the *veg*₂ larva is the poor development of the skeleton. We have seen in Fig. 6 D that *veg*₂, together with presumptive ectoderm, can give a good skeleton for a whole pluteus. In the *veg*₂ larva, however, the skeleton is very poor. This is not because of lack of skeletal material, but for lack of enough animal properties of the ectoderm which has been formed by regulation and which has to

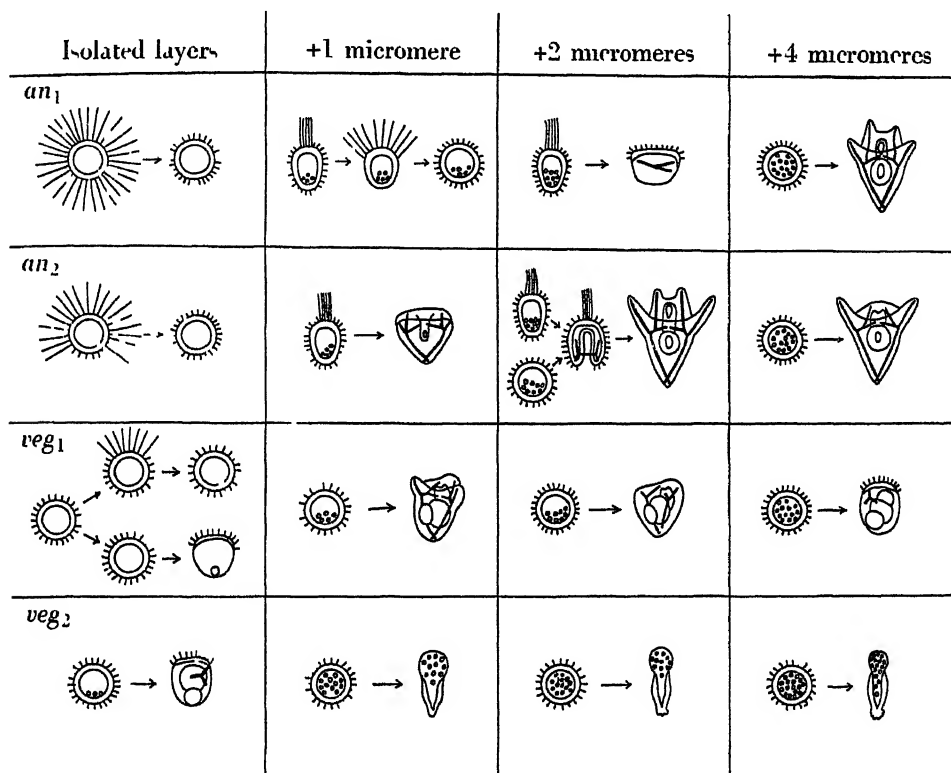


Fig. 9. Diagram of the development of the layers *an*₁, *an*₂, *veg*₁, and *veg*₂ isolated (left column) and with 1, 2 and 4 implanted micromeres. (H-s, 1935.)

lead the outgrowth of the spicules (cf. p. 135). The whole larva is too vegetative to develop a good skeleton and normal ectodermal differentiations. The isolated micromeres first form a small blastula, but at the time when the primary mesenchyme migrates into the blastocoele, the young blastula is dissolved, as the cells wander apart. The micromeres are too vegetative to be able to change the development in a more animal direction. Thus this series demonstrates the decrease of the animal properties (apical tuft) in the fragments *an*₁, *an*₂, *veg*₁, *veg*₂; and the decrease of vegetative characters (skeleton and archenteron) in the fragments micromeres, *veg*₂, *veg*₁ (H-s, 1931, 1935, pp. 359-71).

If we add four, or two, or even only one micromere (H-s, 1935, pp. 371-88) to the just isolated ring of *veg*₂ cells, the larva will be too vegetative to gastrulate (Fig. 9, which refers to equatorial eggs). It forms an exogastrula, of the same type as the exogastrulae obtained after endodermizing a whole egg by means of lithium (Herbst, 1892, 1893, 1896). Four micromeres together with *veg*₁ will give us a larva of the same type as *veg*₂ isolated (Fig. 9). Thus *veg*₁ plus four micromeres seems to have the same constitution with regard to the gradient system as *veg*₂ isolated. In order to get the most pluteus-like larva we have to take *veg*₂ isolated, *veg*₁ plus one micromere, *an*₂ plus two, and *an*₁ plus four micromeres. *an*₁ plus one micromere is particularly interesting. The micromere first checks the apical tuft; later there is what we have called the struggle between the animal and vegetative forces, which results in the defeat of the vegetative forces of the micromere: the apical tuft is larger, no archenteron is induced, and the primary mesenchyme cells are even prevented from producing a skeleton (cf. also 8+0+1; H-s, 1935, p. 334).

The diagram of Fig. 9 has been criticized by v. Ubisch (1936a). But his objections were, it seems to me, anticipated in the original paper by reservations. It was pointed out (H-s, 1935, p. 383) that the diagram only referred to equatorial eggs. The subequatorial eggs were not neglected, for it was described (1935, p. 387) how a diagram for subequatorial eggs would appear. It was emphasized that a diagram of that kind must be very schematic (p. 383). Because of the delicacy of the operations, the material cannot be abundant. As the material is so varied (equatorial and subequatorial eggs with all kinds of transitional types, sometimes in the same batch of eggs) the development most typical for a certain combination will not always be represented by the majority of the cases, when all the results are put together. (For further discussion see H-s, 1938.) I do not doubt the correctness of the principle, expressed in a simplified way in Fig. 9, that differentiation within certain limits depends upon the relative quantities of interacting animal and vegetative material.

If we implant four micromeres in the side of an egg, between *an*₁ and *an*₂ in the 32-cell stage (Fig. 7 B₁), a new vegetative centre is formed, which attracts the primary mesenchyme cells (Fig. 7 B₂) and where an induced archenteron will invaginate, with a more or less complete supplementary skeleton (B₃). The animal-vegetative gradient is illustrated by the fact that, if we implant the micromeres in the animal pole, the induced archenteron will be much smaller, while, when the micromeres are put lower down, between *an*₂ and *veg*₁, it will be larger than those of Fig. 7 B₃, although in the last case the induced, large archenteron will fuse with the normal one, as they stand so close to each other (H-s, 1935, p. 395). Thus, the lower down we put the micromeres, the weaker is the resistance to the micromeres, or, if we put it that way, the more open the material is to the action of the implanted vegetative material (H-s, 1935, pp. 388-98). If instead of micromeres we implant *veg*₂ cells the archenteron is not induced, but formed of the implant, while the skeleton arises from primary mesenchyme cells which have been attracted from the vegetative part of the gastrula (v. Ubisch, 1932a; H-s, 1935, p. 396).

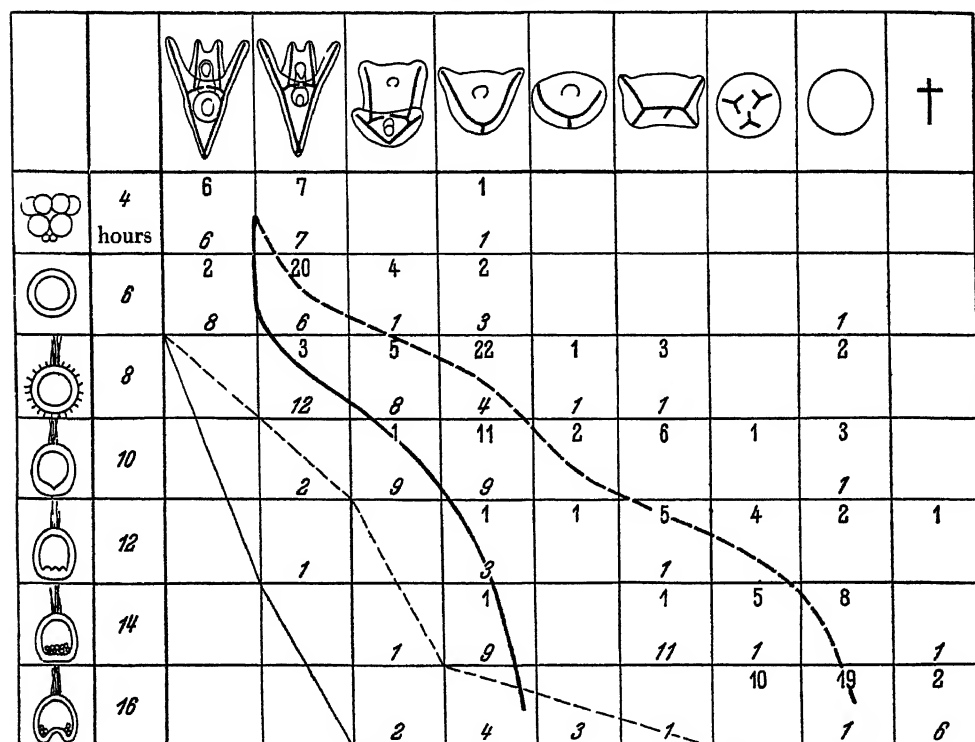
Another experiment showing the struggle between the animal and vegetative

qualities involves the removal of the micromeres from the vegetative pole and their implantation into the animal pole of the same egg (Fig. 7 C_1). In that case we have added nothing to the egg, only translocated the micromeres from one place to another. The result was an enlarged digestive tract and a considerably enlarged coelom (Fig. 7 C_2 ; cf. the control larva C_3). Thus the micromeres could not induce an archenteron, but they weakened the animal forces, allowing the vegetative ones to express themselves farther towards the animal pole (H-s, 1935, p. 411). More endoderm than normally has been formed, although no material was removed or added.

The same explanation holds for some other cases when four micromeres are implanted into the animal pole of an animal half (Fig. 3 C_1 , C_2). The results vary. Sometimes we obtain larvae with two archenterons, opposite each other, one induced at the animal pole by the micromeres, one formed at the most vegetative part of the animal half (Fig. 3 C_3 , C_4). As isolated animal halves never gastrulate (see p. 148), this invagination of the vegetative part of the animal half also seems to be due to a weakening of the animal forces by the action of the micromeres, so that the vegetative forces, normally suppressed by the animal ones, in this region are now strong enough relatively to the animal forces to bring about a gastrulation. The most animal region of these larvae is situated between these two vegetative centres: here we find the apical tuft (Fig. 3 C_3 , C_5). In other cases there may be no invagination at the animal pole, but only at the vegetative side, and in still other cases we find the reverse, thus only at the point of implantation (Fig. 11 e_2 - e_4). A skeleton and stomodaeum may be differentiated in relation to the last-mentioned animal archenteron (Fig. 3 C_6 - C_9). The behaviour of the apical tuft illustrates most clearly and convincingly the rearrangement which takes place. To begin with there may be two vegetative centres, but the original vegetative one is the weaker (in Fig. 3 C_5 shown by the presence of mesenchyme cells and a chalk granule). The apical tuft belongs to the most animal region between these two centres. Then the lower centre is suppressed (the granule is resorbed) and the animal properties are, so to say, pushed down to that region: the apical tuft disappears on the side and a new one is formed at the original vegetative side of the fragment (Fig. 3 C_6 - C_8). All this means that we have in the animal half, as mentioned before, a complete reversal of the polarity of the egg axis, brought about by the micromeres, and of all organs developed in relation to this new axis.

Let us now see how the animal-vegetative determination proceeds in time. Already in the mature, unfertilized egg we have the same segregation as in the early cleavage stages (8-64-cell) just studied, thus the isolated animal half will give only a blastula, with enlarged apical tuft (Boveri, 1901*b*, 1902, p. 83; H-s, 1928*b*, p. 33, 1936*b*). Driesch (1895*a*), Morgan (1895*d*, 1901), and v. Ubisch (1925*b*, 1931*b*, 1932*a*) studied fragments of later stages (blastulae), but their experiments do not tell us much about the progressive determination, as either the plane of fragmentation was unknown or the stage of operation not clearly stated. Jenkinson (1911*a*) removed well-defined pieces from mesenchyme blastulae and gastrulae and was of opinion that fragments of gastrulae still had a certain faculty of regulation, although

less than in earlier stages. A systematic study was undertaken by Hörstadius (1928, pp. 33-41, 1936a). If we isolate animal halves every second hour from the 16-cell stage (4 hr. after fertilization) up to the beginning of the gastrulation (16 hr. after fertilization) (Fig. 10, left column) we find that about 8 or 10 hr. after fertilization (blastula before formation of the primary mesenchyme) there is a change in



towards the animal pole (Runnström (1928*b*) had come to the same conclusion from a study of the action of lithium). The isolated vegetative halves show the different types we knew before (ovoid larva-pluteus) until we get to 14 or 16 hr. after fertilization, when suddenly a new type appears, a pluteus with long anal arms, but entirely without oral lobe. Thus, as regards the vegetative halves too, determination seems to be complete at about 14–16 hr., judging from isolation experiments (H-s, 1936*a*).

If a great number of animal halves are isolated in the 16-cell stage (4 hr. after fertilization) and micromeres are implanted, in some of them immediately, in others 2, 4 and up to 12 hr. later (that is 6–16 hr. after fertilization), we obtain different types of larvae (Fig. 10): typical plutei, plutei with small digestive tracts, larvae with an archenteron that does not reach the stomodaeum, blastulae with ciliated band, stomodaeum, skeleton and arms, the same kind of blastula but without arms, blastulae with skeleton and ciliated band, rounded ciliated blastulae with or without small spicules. These different types occur in a certain specific sequence in relation to the time of implantation. The later the micromeres are implanted—that is to say, the longer the animal halves have been lying isolated before implantation—the less they will be susceptible to the inducing influences of the micromeres (Fig. 10). Six hours after fertilization the plutei will get only a small digestive tract. At 8 hr. few will form gastrulae, the majority become blastulae with skeleton, arms, and stomodaeum. At 14 or 16 hr. we get only ciliated blastulae with small spicules inside. If, however, instead of isolating in the 16-cell stage, we isolate at 4–16 hr. after fertilization and immediately implant the micromeres, we find that the animal halves are able to respond to the induction at a much later stage than in the former case, even at the beginning of gastrulation (Fig. 10). The same difference holds also for the action of lithium on animal halves which have been lying isolated some time before being put into lithium, as compared with those which were isolated at a later stage and immediately put into lithium. Thus the animal half develops more quickly in an animal direction when it is isolated than when it is all the time connected with vegetative material. We note that the determination of a certain stage is not always as complete as one might judge from isolations alone: at a stage when a fragment will self-differentiate the material may still be sensitive to micromere implantation or chemical influences (H-s, 1936*a*; Child, 1936*b*; Lindahl & Stordahl, 1937*b*).

Von Ubisch (1931*a, b*, 1932*a*, 1933*a, b, c, d*, 1934*a, b*, 1935, 1936*b*) implanted micromeres into isolated animal halves at the blastula stage. The larvae developed skeleton and arms. That no archenteron appeared was doubtless due to the late implantation (cf. the paragraph above). In a few cases v. Ubisch (1933*c*, *Echinocyamus* 8 + 0 + 4, 8 + *veg*₁ + 4) obtained beautiful plutei completely devoid of digestive tract. Since in the time series with *Paracentrotus* (see above and Fig. 10) we did not find any plutei having well-developed bilateral skeleton and two pairs of arms in the absence of an archenteron, there seem to be relative differences as to the time of determination in the two species. The formation of blastulae with skeleton and two oral, but only one, median, anal arm, which has often been described (v.

Ubisch, 1932*a*, 1933*c*, 1936*b*; H-s, 1935, p. 333, 1936*a*), is explained by v. Ubisch (1936*b*) in the following manner: the *Anlagen* of the two anal arms are at the time of isolation of the animal half fairly determined and fuse at the closing of the half blastula in the middle line to form one *Anlage*.

If the coelom is removed in one way or another in young echinoderm larvae, a new coelom may be formed atypically: from the endoderm (*Paracentrotus*, Jenkinson, 1911*a*, Runnström, 1917; *Asterias* and *Astropecten*, Driesch, 1895*a*, H-s, 1925*b*, 1928*b*, p. 50; and *Holothuria*, H-s, 1925*c*, 1928*b*, p. 54); from the ectoderm (*Paracentrotus*, Runnström, 1925*a*; *Astropecten* and *Holothuria*, H-s, 1925*b, c*, 1928*b*, pp. 51, 54); or from the mesenchyme (*Astropecten*, H-s, 1925*b*, 1928*b*, p. 50). Larval arms can regenerate (*Luidia*, H-s, 1926). These observations show that regeneration can take place after the completion of embryological differentiation, that the coelom can be formed from other germ-layers than the normal one, and that already differentiated tissues can be redifferentiated into coelom (H-s, 1928*b*, p. 134).

The interactions between animal and vegetative material described above have been most thoroughly studied in *Paracentrotus lividus*. The same phenomena have, however, also been found in other forms, such as *Psammechinus microtuberculatus*, *Sphaerechinus granularis*, *Arbacia pustulosa*, *Echinocardium cordatum* (H-s, 1935, p. 409, 1936*c*). But the determination may differ in some details in different forms (cf. *Paracentrotus* and *Echinocyamus*, p. 161). Also within one species minor differences may occur (equatorial and subequatorial eggs). Moreover, the *Paracentrotus* eggs in the Mediterranean (Naples) and those in the Atlantic (Roscoff) exhibit some differences (H-s, 1935, p. 313).

Von Ubisch (1929, 1931*b*, p. 197, 1933*a*, p. 184, 1933*b*, p. 56, 1936*a*) denies the interaction, described above, between the animal and the vegetative material at the formation of the ciliated band and the stomodaeum, thus maintaining that the animal material develops by self-differentiation. He considers that the animal halves with enlarged apical tuft, developing only to ciliated blastulae, are injured by the operation and the calcium-free sea water. But calcium-free sea water is not poisonous when made of pure chemicals and redistilled water. Von Ubisch does not admit the possibility that halves of equatorial and subequatorial eggs are different. This is strange, since v. Ubisch (1933*c*, p. 63, 1936*a*, p. 629) approves of the explanation of the different behaviour of $8+veg_1+o$ (in most cases without, but some with an archenteron, cf. above p. 151) as being due to a varying position of the furrow veg_1-veg_2 . Von Ubisch has further ignored the fact that the isolated an_1 ring will never form a ciliated band and stomodaeum, as this material normally does. This occurs because the preponderance of the animal properties is so marked in an_1 , whereas in an_2 we have to do with a transitional zone where the vegetative qualities are just beginning to express themselves. It can hardly be maintained that the animal half alone suffers from the treatment, but not the vegetative and meridional halves. It has to be remembered that the more "animal" ("injured" according to v. Ubisch) an animal half is, the more harmonious is the vegetative half from the same egg, and vice versa. New experiments (H-s, 1938) with isolation

of animal halves of *Psammechinus miliaris* in normal sea water (blastomeres of some species are easy to separate without calcium-free sea water) showed exactly the same differentiation as equatorial animal halves of *Paracentrotus*. In one experiment twelve isolated animal halves all had a large apical tuft and later developed into ciliated blastulae. Micromeres were implanted in twelve other animal halves of the same batch of eggs. They *all* developed a ciliated band and a stomodaeum, and also an archenteron (H-s, 1938, Figs. 14-16). This is conclusive proof that the differentiation of equatorial animal halves is not due to a bad condition of the material, since added vegetative material will lead to the formation of the organs in question. The interaction between the animal and vegetative parts is also clearly demonstrated by the constriction experiments (H-s, 1938). Lindahl (1932*b*, 1936*a*, p. 187) has shown that a stretching along the egg axis is enough to cause an enlargement of the apical plate. The same is the case with an early equatorial constriction (Fig. 5 C₁, C₂). Neither ciliated band nor stomodaeum are formed, even if the oesophagus reaches up into the animal half. In Fig. 5 D the most animal third of the egg is partly isolated by the constriction: ciliated band and stomodaeum are lacking in the animal part, which normally gives rise to them. Instead of this they have been formed by regulation in the vegetative component. In Fig. 5 E both partners have developed a ciliated band, but no stomodaeum. The necessity of mutual influences is proved by the constrictions at a *later* stage (Fig. 5 F, G). The interactions took place before the partial or complete isolation, the animal material possessing both ciliated band and stomodaeum (Fig. 5 F₃, G). Because of the weakness of the animal forces in Fig. 5 E the ciliated band is transverse as in lithium larvae (cf. above, p. 145). In Fig. 5 D and G the band is parallel to the egg axis, because in the first case the animal qualities are stronger (the vegetative part comprised two-thirds of the egg), in the second the interaction took place before the thread was more completely constricted.

The regulations which we assume in the gradient system are illustrated in the diagram of Fig. 11. Implantation of micromeres in the animal pole or in the side of an egg creates a new vegetative centre or field, with decreasing intensity in all directions. The new gradient thus established will be smaller at the animal pole, where the resistance is stronger (*b*, *c*). The system $8+0+4$ will resemble that of a typical egg. When the micromeres are placed at the animal pole, we may get two vegetative gradients, the new and the old (e_3), or only one, either the old (e_4) or the new (e_2 , see also Fig. 3). In many cases the regulation seems to aim at a re-establishment of a harmonic larva, e.g. the endodermization in $8+(4+2+2)$ (*i*), $8+2+2$ (*h*), $8+0+4$ (*d*), and the principle of the stronger concentration at the poles in fragments leads to the same effect: $0+4+4$, $0+veg_1+0$, $0+veg_2+0$, $0+veg_2+4$ (*l*, *m*), also $0+4+0$, etc. But this regulation towards a more harmonic differentiation is not universal. The regulation is subject to quantitative rules. The endodermization when relatively too little presumptive endoderm is present ($8+(4+2+2)$, $8+(8+4+4)$, $8+2+2$, $8+0+4$, etc.) is proportional to the vegetative material—note the series $8+2+2-8+1+0$ (Fig. 8)—and when the vegetative forces are particularly reduced they may become suppressed: $8+\frac{1}{2}+0$ (Fig. 11 *n*, 8 E),

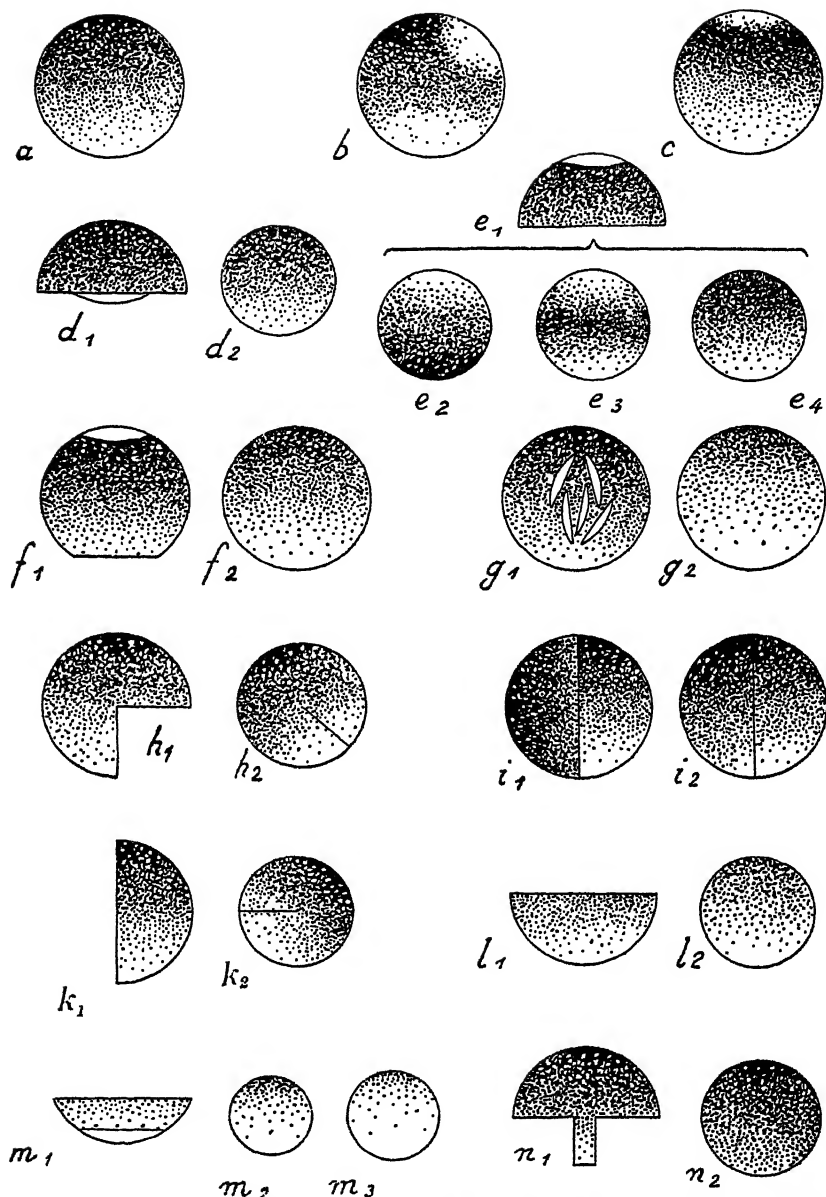


Fig. 11. Diagram of the hypothetical changes in the gradient system of larvae of different compositions. Animal gradient black, vegetative gradient white. *a*, normal egg. *b*, implantation of four micromeres between an_1 and an_2 (cf. Fig. 7 B). *c*, the same in the animal pole. *d*₁, implantation of four micromeres in the vegetative part of an animal half. *d*₂, the gradient system after rearrangement (cf. Fig. 6 F). *e*₁, implantation of four micromeres in the animal pole of an animal half. Later an archenteron may appear at the animal (*e*₂), the vegetative (*e*₄), or at both poles (*e*₃) (cf. Fig. 3 C). *f*₁, *f*₂, predominance of the vegetative qualities following translocation of the micromeres of an egg to its animal pole (cf. Fig. 7 C). *g*₁, *g*₂, the same after the filling of a cleavage stage of an entire egg with micromeres (cf. Fig. 8 G). *h*₁, *h*₂, the experiment 8+2+2 (cf. Fig. 8 A). *i*₁, *i*₂, fusion of an animal and a meridional half (cf. Fig. 7 A). *k*₁, an isolated 1/2-blastomere. *k*₂, polarity and gradient system after the half blastula has closed (cf. Fig. 3 B). *l*₁, *l*₂, an isolated vegetative half (cf. Fig. 5 B). *m*₁, the layer $veg_2 + 4$ micromeres ($o + veg_2 + 4$). *m*₂, the isolated veg_2 layer. *m*₃, $o + veg_2 + 4$ (cf. Fig. 9). *n*₁, *n*₂, in 8+1/2+0 (i.e. an animal half+a half macromere) the presumptive archenteron may be suppressed (cf. Fig. 8 E). (H-s, 1935.)

$8+0+1$, an_1+0+1 (Fig. 9). On the other hand, a surplus of vegetative forces also causes an endodermization: the enlarged archenteron in vegetative halves, the vegetative type of $8+0+12$, $8+4+24$ (Figs. 11 g, 8 G). Thus the vegetative forces cause an endodermization when increased, or when decreased to a certain degree. If they are considerably decreased, they may be suppressed. They also express themselves stronger than normally if the animal forces have been weakened at the animal pole: the vegetative archenteron in animal halves with animal micromeres (Figs. 3 C, 11 e_3 , e_4), an egg with the micromeres translocated to the animal pole (Figs. 7 C, 11 f). In these cases we have to deal, not with a local effect at the point of implantation, but an action at distance, thanks to an effect on the system as a whole. In animal fragments such as an_1 rings and equatorial animal halves we do not notice any obvious stronger concentration of the vegetative properties. The animal properties predominate. And yet the vegetative ones are strong enough to bring about gastrulation, provided the animal forces be reduced by micromeres in the animal pole of the animal half (Figs. 3 C, 11 e_3 , e_4). In vegetative halves we find an endodermization and at the same time a concentration of the animal properties (possibly apical tuft, mouth, etc.). In still more vegetative fragments the endodermization yields to an ectodermization, as a result of reconcentration ($0+veg_2+4$); but, on the other hand, the frequently poor condition of the exarchenteron may be a sign of a too strong vegetative concentration.

All these results indicate that we have not to reckon with specific factors for every organ, but only with two different gradients. The relative amounts of the animal and vegetative forces determine the development. The reduction or lack of interaction in stretched or constricted eggs makes it probable that normally diffusion processes take place between the animal and vegetative parts. The reconcentration in some fragments seems to indicate an excess or a deficit compared with the presumed original content of animal and vegetative substances (e.g. the suppression of the presumptive endoderm in some $8+1/2+0$, the ectodermization in $0+veg_2+4$, etc.). Lindahl (1936 a, p. 338-54) has advanced a physiological explanation of the regulations in fragments. He has found two different types of metabolism in the sea urchin's egg (1933, 1934, 1935, 1936 a, b, 1938; Lindahl & Öhman, 1936, 1938; Lindahl & Stordahl, 1937 b): an animal type with increasing oxygen consumption, capable of being checked with lithium and probably connected with a breakdown of carbohydrates, and a vegetative, characterized by a breakdown of proteins. In connexion with these two types of metabolism, which are based on differences in the animal and vegetative material, animal and vegetative substances are formed, and they affect the differentiation. They are distributed by diffusion and are used up. Their formation is a reversible process, and they may suppress each other. In fragments, and after transplantations, the conditions for formation and diffusion are different and may explain the results obtained in the experiments.

The existence of two types of metabolism along the egg axis and the development of the animal and vegetative organs in some of our larvae (e.g. the pushing away of the apical tuft from a central region to the most vegetative side under the

influence of micromeres implanted at the animal pole, Fig. 3 C₅, C₆) makes it difficult to assume, with Child (cf. above, p. 147, and also v. Ubisch, 1938), that there is only one gradient along the egg axis.

It is more likely that we have to reckon with only quantitative differences along the dorso-ventral and left-right axes, as a weakening of the presumptive ventral and left side respectively will result in the formation of ventral and left organs on the presumptive dorsal and right sides respectively (cf. above, pp. 144, 146 and 147).

When the strong inducing effect of the vegetative material was first demonstrated (H-s, 1928 *a*, *b*), an organization centre in the vegetative part of the egg was spoken of (cf. also Boveri above, p. 147). Further investigations have revealed the importance of other centres or gradients too, namely, on the animal, the ventral, and the left side. We seem to have a system with three axes, each axis with its two poles differing from each other (Runnström, 1918). The differences may be qualitative or quantitative in nature. The question whether the ventral centre is formed because of a preponderance of the animal and vegetative gradients of this side (Schleip, 1929, p. 539; Runnström, 1929; Lindahl, 1932 *b*) is, so far, difficult to answer (cf. H-s, 1938, pp. 246-8).

A comparison with the amphibian egg has been made in the following way (Lindahl, 1932 *b*, p. 320, 1936 *a*, p. 355; H-s, 1938, pp. 248-53). Some recent results indicate that in the amphibian egg we have analogies to the animal-vegetative system in the sea urchin. Isolated parts of the presumptive dorsal lip of the blastopore of the amphibian egg may give more differentiation than the prospective significance of the material would suggest (Holtfreter, Lopaschov, Shen). Just as organizer material may affect presumptive ectoderm, the latter is capable of changing pieces of the dorsal lip into epidermis or neural plate (Vogt, Mangold, Bruns, Lopaschov, Töndury). These phenomena may have their analogies in the animal and vegetative inductions and suppressions in the sea urchin's egg. Penners has found that an invagination may also take place in the animal half of an amphibian egg, if a part of the yolk comes into contact with the surface, as implanted micromeres induce an archenteron. Because of its importance for the appearance of bilateral symmetry, Lindahl compares the ventral centre of the sea urchin with the dorsal of the amphibian. Beside this correspondence, there are also considerable differences. The ventral centre, for example, is not stable in its position, a new centre is readily formed on the opposite side, and no inducing power has been met with. Furthermore, we must remember the existence of a centre at the left side, which, like the ventral one, seems to inhibit the formation of a similar centre on the opposite side (H-s, 1925, 1928 *b*), and which is of particular importance for further development, as the appearance of the hydrocoele is essential for the determination of the axis of the radially symmetrical sea urchin and for the development of the ectodermal part of the sea urchin rudiment (Runnström, 1917, 1918; v. Ubisch, 1929).

On the other hand, Dalcq & Pasteels (1937), although considering the animal-vegetative system of the sea urchin a binary one, assume the animal-vegetative gradient of the amphibian egg to be simple, and they compare the animal gradient of the sea urchin with the amphibian dorso-ventral field.

VII. DETERMINATION OF THE BILATERAL SYMMETRY

The determination of the bilateral symmetry has in part been treated in connexion with the determination of the position of the dorso-ventral axis. We have seen that the axis remains in the right, left, and ventral halves, but is reversed in the dorsal halves and that it coincides with the stretching axes in eggs stretched at right angles to the egg axis.

The bilateral organization in an early cleavage stage (2-64-cell stages) is demonstrated by the fact that isolated meridional halves show deficiencies as regards skeleton and arms (Runnström, 1914; Plough, 1927, 1929). A detailed analysis showed the following (H-s & Wolsky, 1936; H-s, 1936a). Right and left halves are generally slightly defective on the cut side. The ventral halves develop their ventral side faster and better than do the dorsal ones, in which the ventral side is developed on the former dorsal side (Fig. 4 A₂). The later we undertake the separation, the more marked are the deficiencies. For example, if a blastula is divided into right and left halves 10 hr. after fertilization, we shall find only a small spicule on the cut side when the skeleton on the other side is already well developed. At the beginning of gastrulation (16 hr.) the determination has proceeded so far that each lateral half differentiates exactly as a half of a pluteus, each with only one piece of skeleton and two arms. At about the same time the dorso-ventral axis becomes fixed; after isolation in the late blastula stages it cannot be reversed any more. At 10 and 12 hr. there may appear some intermediate stages, showing more or less pronounced traces of a ventral side on both the original ventral and dorsal sides. At the beginning of gastrulation the dorsal and ventral halves also differentiate exactly as the corresponding halves of a pluteus.

Marx (1931), who removed two blastomeres at the 8-cell stage (4+4+4, 8+2+2, 6+3+3), or two micromeres at the 16-cell stage, observed marked deficiencies and concluded that at this early stage the egg has a rather determined bilateral organization. However, Hörstadius (1935, p. 454) and Hörstadius & Wolsky (1936) obtained in nearly all cases perfectly symmetrical skeleton and arms from cleavage stages of the same composition. Thus we may draw conclusions as to the bilateral organization from isolated 1/2-larvae (and 1/4-larvae); but if only a quarter of the egg is removed, it has the power of complete regulation.

Driesch (1892, 1906), Boveri (1907), and Plough (1929) isolated all the four 1/4-blastomeres from one egg. Boveri reports four plutei in some cases. If the four blastomeres are isolated in such a way that it is known which of the cells have been neighbours to each other, we often find in the development of these quartets that two of the larvae, evidently the ventral ones, develop their ventral side faster and better than do the other two, which we designate as dorsal (H-s & Wolsky, 1936). In other cases we find one ahead of the other three, or one decidedly the best, whereas the opposite one develops still more slowly than the other two. In these cases we obviously have to deal with one ventral, one right, one left, and one dorsal blastomere. The great majority of the quartets thus indicate a bilateral organization in the early cleavage stages.

If an egg is divided into eight meridional parts (with the exception of the micromeres which have first to be removed), we find that such small fragments may give rise to plutei (H-s & Wolsky, 1936). Whether some of these small larvae which were isolated in the right order of sequence correspond to the ventral, others to the dorsal side, is not easy to determine, as the fragments are generally rather irregular. But it is interesting to note that a fragment of such small size may form a real pluteus. (The smallest fragment that has been found to gastrulate is a half macromere (H-s, 1936*b*; see also Schleip, 1929, pp. 432-8).)

The old problem of whether the egg is bilaterally organized before fertilization can be approached by dividing the egg into meridional halves (orienting with the aid of the pigment band in *Paracentrotus*) and subsequently fertilizing the two halves. One will of course be haploid. The haploid halves are slower than the diploid in their development and also smaller. Some pairs show complementary deficiencies on the right and left sides. Others differentiate like ventral and dorsal halves. There are cases where the diploid partner is the better developed larva (designated as ventral), but also cases where the haploid partner clearly shows itself to be ventral, although smaller and slower than the diploid one, thus indicating a bilateral organization before fertilization (H-s & Wolsky, 1936). Lindahl (1936*a*) assumes only a slight preformation in the unfertilized egg, the ventral centre being gradually formed during early development.

The isolated, most animal (an_1) and most vegetative fragments (micromeres, $veg_2 + 4$) do not show any bilateral differentiation, but if fused form typical plutei (H-s, 1935, p. 430). This does not necessarily mean that they lack a bilateral organization, as this may (e.g. in an_1) be concealed under the uniform animal differentiation (apical tuft, later thick ciliated epithelium all round the blastula).

The differentiation of fragments is generally slower than that of whole eggs. This retardation has always been supposed to be due to a regulation which requires time (Driesch, 1900*a*, 1906, 1908*a*; H-s, 1928*b*, p. 65; H-s & Wolsky, 1936, p. 85). We have witnessed the reversal of the dorso-ventral axis in dorsal halves, which implies a regulation requiring time, as the ventral and dorsal halves are of exactly the same size, but the latter are slower in differentiation. We have seen the same phenomenon in animal-vegetative fragments, e.g. the isolated veg_1 (p. 156). But Tyler (1933, 1935) refers the retardation to energy conditions, not to regulation. He has shown that the smaller a larva is, the more energy is required to bring it to a certain stage of differentiation, and also that giant eggs composed of two fused eggs develop faster than normal ones, although we must presume that regulation is taking place in them. It is possible, however, that both views may be combined, namely, that there is a general delay in fragments due to energy conditions and, moreover, a specific delay, more marked in dorsal fragments than in ventral, more marked in 1/4-larvae than in 1/2-larvae, due to regulation (H-s & Wolsky, 1936, p. 108).

VIII. XENOPLASTIC IMPLANTATIONS AND TRANSPLANTATIONS

Von Ubisch (1931-9) and his school (Nümann, 1933; Altrogge, 1935; Schmidt, 1936) implant micromeres from one species into the blastocoel of an entire egg, or an animal half, or an egg without micromeres, the host belonging to another genus or even order of sea urchins. As the primary mesenchyme (skeleton cells), which is derived from the micromeres, can be considered as a germ-layer, von Ubisch designates the larvae as germ-layer chimaeras (*Keimblattchimären*). Micromeres are able not only to live in a host of another genus or order, but also to interact with the host, leading to the formation of more or less typical skeleton and arms (cf. above, p. 135). The character of the rod, simple or fenestrated, is determined by the primary mesenchyme cells. The ectoderm of the host seems, upon the whole, to determine the position of the skeleton-forming cells and the outgrowth of the rods (cf. p. 135) in conformity with its species characters. A detailed presentation of the results by von Ubisch and his school is omitted here, since they were reviewed by von Ubisch himself in the last number of *Biological Reviews*.

In many cases the skeletons of chimaeras with mixed skeleton cells (foreign micromeres implanted into an entire egg) are of the same type in reciprocal combinations. Moreover, they greatly resemble the skeleton in hybrids (although on the whole the influence of the father in hybrids seems to be stronger than that of the donor in germ-layer chimaeras (Schmidt, 1936, p. 248)). Von Ubisch (1933*b*, *d*, p. 83) maintains that there is no difference if the realization of the genes is due to heterozygous nuclei (in hybrids), or to homozygous nuclei from different species working in a common cytoplasm, the skeleton-forming syncytium. In order to study the effect of nucleus and cytoplasm, von Ubisch (1934*a*, *b*, 1935) varied the amount of nuclear material of one species in relation to nucleus and cytoplasm of the other by implanting micromeres of a hybrid into a whole blastula of the same species as the mother of the hybrid, or into blastulae with 1-4 micromeres removed. The hybrid micromeres in some crosses live better in the host than in the hybrid itself. As these hybrid chimaeras (*Bastardchimären*) are composed of cytoplasm of only one species but have chromatin from both, in varying proportions, and as the character of the skeleton on the whole varied according to the amount of chromatin present, von Ubisch (1933*d*, 1934*a*) concludes that the cytoplasm has no influence on the species character of the skeleton. I do not find this conclusion convincing. First, the skeleton in chimaeras is so variable that a faint cytoplasmic influence, if there be any, could hardly be detected. Secondly, the new observations by von Ubisch (1937*a*) on the formation of the skeleton in normal larvae and in larvae with implanted vitally stained micromeres seem to show that the formation of the chimaera skeleton is a very complicated process. In some parts of the larva where skeleton cells of only one species are present the rods will be typical of that species, thus without influence from the foreign nuclei in that part of the syncytium (the ectoplasm of the skeleton cells fuses). In other parts the skeleton will be formed by a syncytium probably under the mixed influence of the two kinds of chromatin. Thus a real intermediate skeleton will probably arise in some parts.

But as the cells move, so that the formation of a rod may be initiated by certain cells (as part of the syncytium) but completed by others (v. Ubisch, 1937*a*), we have to reckon with the possibility that the rod is first secreted by a mixed syncytium and then completed by a syncytium with nuclei only of one species, and so on in different combinations. Thus the skeleton of the chimaeras must be a mosaic of species-specific parts and parts of mixed origin, and in the latter case we probably have to deal partly with a simultaneous action of the two nuclear components in a common syncytium, partly with a successive action of qualitatively different parts of the syncytium, thus what might be called a "mosaic in space and time". It seems doubtful to me whether under these circumstances a direct comparison can be made between hybrids and chimaeras, and whether any binding conclusion as to a possible faint cytoplasmic influence can be deduced from an organ system with such complexity of origin and variety of result. That the species character of the donor should predominate the more the skeleton cells of the host are removed is what would be expected.

Von Ubisch (1932*b*) and Nümann (1933) have found a greater variation of the skeleton at higher temperatures, and also with some chemicals, which lower the viscosity of the cytoplasm (KOH, NaI, NaSCN, v. Ubisch, 1937*b*, 1939). As a result, a skeleton of the simple type may acquire an appearance more like the complex type. Von Ubisch (1937*b*, 1939) explains the directed variation, that leads to formation of a more complex skeleton, as being due to a lowering of the viscosity of the cytoplasm by the heat and chemicals. He assumes that differences in viscosity largely, although not completely, account for the differences in skeleton in different species. In hybrids (with cytoplasm of only one species) the intermediate skeleton is said to be due to the genes, acting upon the cytoplasm. In germ-layer chimaeras the intermediate skeleton is ascribed to the action of the two different kinds of cytoplasm mixed in the syncytium. It is not quite clear how this view can be brought into agreement with the earlier conclusions (cf. above) that there is no difference in the realization of the genes in hybrids (with heterozygous nuclei and one kind of cytoplasm) and chimaeras (with two kinds of homozygous nuclei and two, more or less mixed cytoplasm), and that the species character is due entirely to the nucleus, not to the cytoplasm.

The whole eggs with implanted micromeres are not pure germ-layer chimaeras, since the primary mesenchyme is mixed. Also if the micromeres of the host are removed before implantation, we may get a mixed set of skeleton cells, for the host may form such cells by regulation (see above, p. 151). A pure chimaera was obtained when von Ubisch implanted foreign micromeres into animal halves, but the endoderm was missing, the implantation in the blastula stage evidently being too late to effect induction of an archenteron (see p. 161). Von Ubisch (1931*b*) tried to make heteroplastic transplantations in early cleavage stages, but the larvae did not live longer than to the gastrula stage. Hörstadius (1936*c*, p. 860) succeeded in fusing the presumptive ectoderm ($8 + veg_1$) of *Psammechinus* with the presumptive ento- and mesoderm ($veg_2 + 4$) of *Paracentrotus*. As the larvae of these species are very similar, the chimaera does not differ notably from any of them. Bierens de

Haan (1913 *a, b*) obtained random ectodermal fusions of *Psammechinus* and *Paracentrotus*. Hörstadius (1936*c*) got a unit, a single pluteus, from two meridional halves of the same species. In the presumptive ectoderm of *Psammechinus* were implanted (H-s, 1936*c*) micromeres of *Sphaerechinus*. Although originating from another family, the micromeres were able to induce an archenteron. The skeleton showed, strange to say, a rather intermediate type, but we do not know for certain whether the host formed some skeleton cells by regulation (cf. H-s, 1936*c*, p. 865).

Several investigators have tried to rear larvae from enucleated eggs, fertilized with sperms from another species (heterosperm merogones, Boveri, 1889, 1914, 1918; Taylor & Tennent, 1924; Fry, 1927; Harvey, 1933*a*), but the larvae all died at an early stage. But *Psammechinus microtuberculatus* cytoplasm + *Paracentrotus*

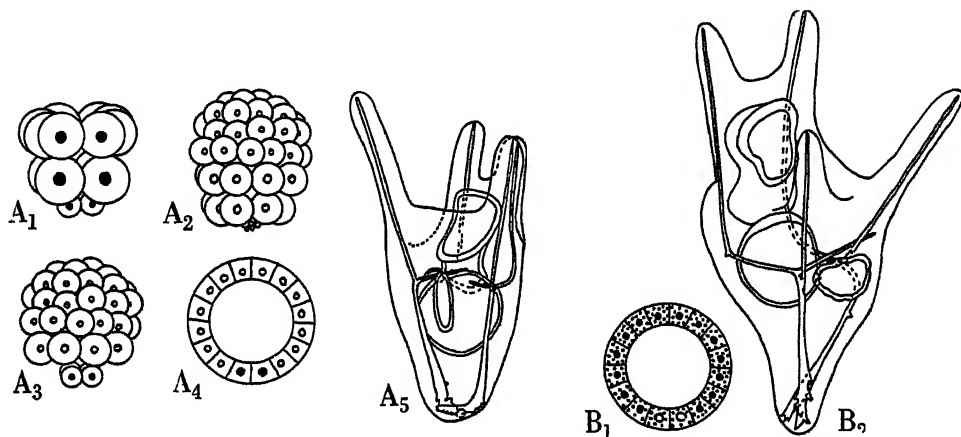


Fig. 12. *A*₁, diagram of a 16-cell stage of a heterosperm merogone *Paracentrotus lividus* cytoplasm (white) + *Psammechinus microtuberculatus* nucleus (black). *A*₂, a 64-cell stage of *Paracentrotus* (nuclei white). *A*₃, the presumptive ectoderm (animal half + *veg*₁) of *Paracentrotus* combined with the micromeres of the merogone. *A*₄, the blastula consists of *Paracentrotus* cytoplasm and nuclei, except for the nuclei of the micromeres = the presumptive skeleton-forming cells. *A*₅, these nuclei give the pluteus the appearance of a *Psammechinus* larva (the thickened, bent, irregular clubs of the body rods). *B*₁, the reciprocal combination. *B*₂, although the larva consists of *Psammechinus* cytoplasm (dotted in *B*₁) and nuclei, except the nuclei of the skeleton-forming cells, the pluteus looks like a *Paracentrotus* larva (straight, slender body rods). (H-s, 1936*c*.)

lividus nucleus (sperm) as well as the reciprocal combination gave plutei which swam and fed for a couple of weeks, and they also showed species characters (H-s, 1936*c*). The positive character, the bent, thickened, irregular club of the body rods in *Psammechinus*, was found to follow the nucleus in *Paracentrotus* cytoplasm + *Psammechinus* nucleus. In the reciprocal combination, where we should expect the straight, slender, smooth clubs of *Paracentrotus*, we also find some irregularities, but it is impossible to decide whether they are due to the cytoplasm of *Psammechinus* or the nucleus of *Paracentrotus* (see H-s, 1936*c*, p. 853-7). Germ-layer chimaeras were made with the presumptive ectoderm of one species combined with micromeres from a heterosperm merogone (Fig. 12). An archenteron was induced. Although the one species was represented only by the nuclei of the skeleton-forming cells (Fig. 12 *A*₄, *B*₁) the species character, i.e. the clubs of the

body rods, was developed in conformity with the character of that species (Fig. 12 A₅, B₂) (H-s, 1936c, p. 844).

The sea urchin's egg has thus in one respect proved excellent material for an investigation of the role of nucleus and cytoplasm. The larvae with nucleus of one species and cytoplasm of another lived long enough to develop a species character. In von Ubisch's chimaeras we have a closer connexion between nuclei and cytoplasm of different species than in other animals, with the exception of hybrids. In both cases the nucleus obviously has a positive effect on the species character. It is to be regretted that the characters in question are not defined sharply enough to permit of a conclusion as to the possible role of the cytoplasm.

IX. SUMMARY

In the cleavage stages the sea urchin's egg can be divided into five transverse layers, an_1 , an_2 , veg_1 , veg_2 , and the micromeres (Fig. 1). The ectoderm of the pluteus larva is derived from $an_1 + an_2 + veg_1$, while veg_2 gives rise to the secondary mesenchyme, the coelom, and the endoderm. The micromere material migrates into the blastocoele before gastrulation, forming the primary mesenchyme, which produces the skeletal rods. The position of the skeleton-forming cells and of the rods is determined by the ectoderm.

The factors determining the cleavage type of the 16-cell stage (8 meso-, 4 macro-, and 4 micromeres) seem to be (1) progressive changes in the cytoplasm, causing spindles formed a certain time after fertilization to lie in a certain direction, (2) the presence in the vegetative part of the egg of a region of micromere-forming material, and (3) the activation of that material a certain time after fertilization. This leads to partial cleavage of isolated blastomeres, and to whole, intermediate or partial cleavage of fragments of undivided eggs, depending upon the time and plane of isolation. Whole eggs may also show partial cleavage. Differentiation is independent of the type of cleavage which the egg or fragment has undergone.

The polarity (animal-vegetative axis) of the egg is fairly stable, since it is not altered by centrifuging or by moderate stretching, and it is more or less retained in fragments. On the other hand, the polarity can be changed both by a greater degree of stretching and by placing animal and vegetative material in atypical relationship to one another. A new axis may then be induced, and the whole polarity may sometimes be reversed. A reversal can be brought about both by vegetative and by animal material. The dorso-ventral axis is less stable, as it adjusts itself in accordance with the direction of stretching (perpendicular to the egg axis), not only to a considerable, but also to a moderate degree of stretching. After considerable stretching or constriction, both of which involve partial physiological isolation, as well as on complete isolation, the dorso-ventral axis is spontaneously reversed in the dorsal half. In right and left halves the dorso-ventral axis is maintained, and the larvae are more or less defective on the cut side. In starfish larvae a similar reversal of the right-left axis may occur in right halves.

After isolation animal material will form a considerably enlarged apical tuft and

later develop only into ciliated cylinder epithelium. In *veg*₁, *veg*₂, and the micromeres, we find the faculty of checking the enlargement of the apical tuft and of causing the formation of stomodaeum, ciliated band, and pavement epithelium out of the animal material. Moreover, *veg*₂ has the faculty of forming endoderm and skeletal cells, and under certain conditions also ectoderm. An endodermization of presumptive ectoderm can also be brought about by *veg*₂, but this power of induction is much stronger in the micromeres. To explain the conditions in the sea urchin's egg we assume an animal and a vegetative gradient, both reaching the opposite pole and progressively diminishing. The animal and the vegetative qualities or forces have to interact in order to bring about normal differentiations, e.g. vegetative influences are necessary for the formation of ciliated band and stomodaeum, animal ones for gastrulation and skeleton formation, and so on. The differentiation depends—within wide limits—upon the relative amounts of animal or vegetative material present. We find an endodermization both when the vegetative material is relatively increased (vegetative halves) and decreased ($8+2+2$), provided the decrease is not too extreme. In this last case ($8+1/2+0$) the vegetative properties may be suppressed. But thanks to a reconcentration at the poles of fragments, the animal and vegetative forces are often able to express themselves more strongly than would be expected from the prospective significance of the material or from the amounts present (apical tuft and mouth in vegetative halves, ectoderm in isolated *veg*₂, as animal differentiations; skeletal cells in $8+4+0$, $0+4+0$, $0+veg_2+0$, as vegetative differentiations, and so on).

Implanting micromeres of one species into the blastocoele of another, von Ubisch has studied the formation of the skeleton in such germ-layer chimaeras (see the article by von Ubisch in *Biological Reviews*, vol. 14, 1939, p. 88). The roles of nucleus and cytoplasm in the formation of the skeleton have also been studied in heterosperm merogones (larvae with nucleus of one species and cytoplasm of another). A species character was found to follow the nucleus. Both in the germ-layer chimaeras and in the merogones the conditions are too complicated to allow a conclusion as to any possible role of the cytoplasm.

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TYPEN DER ARTBILDUNG

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(Received 12 August 1938)

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I. EINLEITUNG

DER Vorgang der Evolution ist so komplex, dass er nur mit Hilfe verschiedener Sonderdisziplinen analysiert werden kann. Paläontologie, Genetik, Systematik, vergleichende Anatomie und Embryologie bzw. Entwicklungsmechanik müssen ihre Erkenntnisse miteinander verbinden, um die Formenumbildung in ihrem Gesamtablaufe verständlich zu machen und um zugleich auch der Verschiedenartigkeit der Entwicklungswege gerecht zu werden. Neben den fundamentalen Ergebnissen der Vererbungsforschung hatten nun aber die Untersuchungen der Systematiker lange Zeit verhältnismässig wenig Beachtung gefunden. Man hielt es vielfach für ausreichend, den Vorgang der Artbildung an einzelnen Beispielen intensiv genetisch und entwicklungsgeschichtlich zu studieren und die gewonnenen Resultate zu verallgemeinern. So konnte die Formumwandlung zwar weitgehend geklärt werden, aber es war nicht möglich zu überblicken, wie weit die ermittelten Evolutionsvorgänge *als Norm* gelten können. Ein solches Urteil kann erst auf Grund taxonomischer Untersuchungen an ganzen Formengruppen gefällt werden.

Dabei hat es sich nun tatsächlich herausgestellt, dass wir *verschiedene Typen der Artbildung* unterscheiden müssen. Abgesehen von der Art der zugrundeliegenden Mutation selbst (Genmutation, Chromosomenmutation, Poly- und Heteroploidie, plasmatische Änderungen; Dominanzverhältnisse) kann die Art der Isolation eine sehr verschiedene Wirkung haben, es kann eine *Auslese* wirksam sein oder nicht, die Auslese kann zu einer *richtungslosen* oder zu einer *gerichteten* (orthogenetischen) Weiterbildung führen, und es kann sich schliesslich um Wandlung einzelner Merkmale oder um *ganzheitliche Strukturänderungen* handeln. Es ist deshalb wohl angebracht, einmal vom Standpunkte zoologischer Systematik solche verschiedenen Artbildungstypen aufzustellen und die Häufigkeit ihres Vorkommens in einzelnen Tiergruppen zu beleuchten. Nun zwingt die unübersehbare Formenfülle und die fast ebenso unübersehbare systematische Literatur natürlich zur Beschränkung auf einige Gruppen. Der Verfasser möchte deshalb in erster Linie seine eigenen entsprechenden Untersuchungen an Vögeln, Säugetieren und Landschnecken zusammenfassen.

II. RICHTUNGSLOSE ARTBILDUNG

Allgemein wird die Mutation heute als Folge der begrenzten Stabilität der Gene angesehen. Sie kann deshalb erbliche, Abweichungen praktisch aller morphologischen und physiologischen Merkmale hervorrufen, wie dies auch die zahllosen genetischen Analysen von Tieren und Pflanzen erkennen lassen. *Die Mutation ist mithin im allgemeinen richtungslos*, und sie führt zunächst nur zu einer Störung des vorhandenen, im Laufe der Evolution erworbenen biologischen Gleichgewichtes. Über mehrere Generationen hinweg können nun natürlich nur die verhältnismässig wenigen mutativen Abweichungen erhalten bleiben, die physiologisch unschädliche Änderungen bedingen und die speziell Vitalität und Fertilität nicht nachteilig beeinflussen. Wenn somit eine erste natürliche Auslese nur einen geringen Prozentsatz von Mutanten fortbestehen lässt, so können diese aber immer noch die verschiedensten morphologischen und physiologischen Merkmale betreffen. So entsteht das bekannte Bild erblicher individueller Variabilität aller Einzelcharaktere.

Kann nun schon diese richtungslose individuelle Variabilität zur Rassen- und Artbildung führen? Offenbar ist dies durchaus möglich. Ein verhältnismässig geringer Teil der mutativ auftretenden Merkmale verhält sich in seinem Erbgange mehr oder minder stark *dominant*. Derartige Varianten werden also im Laufe der Zeit auch dann in einer Population immer mehr vorherrschend werden, wenn keinerlei Selektion eintritt. Entsprechende Beispiele sind aus der Vogelwelt bekannt (vgl. Stresemann, 1926). Der neuseeländische Fliegenschnäpper *Rhipidura flabellifera* besitzt eine melanotische Mutante (Abb. 1), bei der das gesamte Gefieder stark verdunkelt ist. Diese Form war ursprünglich nur von der Südinsel Neuseelands bekannt, wurde aber seit 1864 auch auf der Nordinsel beobachtet, wo sie schnell an Zahl zunahm und heute schon bis zum Nordzipfel hin gefunden wird. Es ist zu erwarten, dass die Normalform nach einigen Jahrzehnten völlig verschwunden sein wird. In entsprechender Weise hat eine melanistische Variante des

Zuckervogels *Coereba saccharinum* auf den Antilleninseln St. Vincent und Grenada in den letzten Jahrzehnten die Ausgangsform weitgehend verdrängt. Es liegt nahe, das Vorherrschen ähnlicher melanotischer Mutanten des Webefinken *Colius passer ardens* in weiten Gebieten West- und Central-Afrikas, des Würgers *Lanius schach schach* in Südchina, der Grassmücke *Sylvia atricapilla heineken* auf Madeira und den Canarischen Inseln, sowie auch das bereits ausschliessliche Vorkommen der weissen Mutante des australischen Habichts *Accipiter nov. novae-hollandiae* in Tasmanien ähnlich zu deuten. In all diesen Fällen ist die Abweichung der Mutanten so stark, dass sie als scharf differenzierte Rassen angesprochen werden können, d.h. die dominanten Mutanten führen zu einer Rassenwandlung. Allerdings ist bisher noch nichts darüber bekannt, ob nicht etwa Vitalität oder Fertilität der Mutanten gegenüber der Normalform gesteigert ist. (Das Auftreten solcher Varianten bei sehr verschiedenen Vogelgruppen macht dies indess nicht wahrscheinlich; doch wäre wenigstens die leicht mögliche Prüfung der Fertilität wünschenswert.)

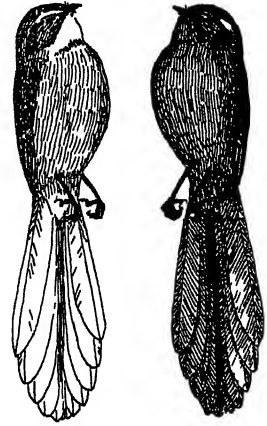


Abb. 1. Fliegenschnäpper *Rhipidura flabellifera* und seine melanotische Mutante (nach Stresemann, 1926).

Es gibt nun im ganzen Tierreich eine grosse Zahl geographischer Rassen, deren Entstehung mit einiger Wahrscheinlichkeit auf das gleiche Schema zurückgeführt werden kann. Zumindest sind jedenfalls die charakterisierenden *Hauptmerkmale* bei den einzelnen Formen entsprechend derart "regellos" abgeändert, dass die Wirkung einer Selektion nicht erkennbar ist oder dass die Selektion bei jeder Rasse ein anderes Gen trifft, also für den ganzen Rassenkomplex ebenfalls richtungslos ist. Unter den Vögeln sind es besonders bunte Tropenformen, bei denen eine Beziehung ihrer Rassenmerkmale zu Auslesefaktoren nicht festzustellen ist.

Als Beispiel sei hier eine Gruppe bunter Fruchtauben (*Ptilinopus*) des australisch-polynesischen Gebietes (Abb. 2) genannt, die ich schon 1926 einmal zusammenstellte (Rensch, 1929a). Es handelt sich um 20–30 Formen, die durch den Besitz einer violetten, rötlichen oder blauen Kopfplatte mit gelbem Saum ihre enge Verwandtschaft anzeigen (daneben andere gemeinsame Merkmale) und die sämtlich einander geographisch ersetzen. Dabei ist die Färbung der einzelnen Formen im übrigen denkbar verschieden: das Bauchgefieder z.B. ist entweder grün oder gelb oder orange mit einem violetten, blauen, grauen oder fehlenden Mittelfleck, der Nacken ist gelbgrau, blaugrau oder dunkelgrün, der Schwanz kann eine gelbe oder weisse Endbinde haben oder eine solche fehlt usw. Verschieden ist weiterhin die Grösse, die Flügel-Schwanz-Relation (Schwanz z.B. bei *coronulatus* von Neu-Guinea 57 % des Flügels, bei *xanthogaster* von Banda-Timorlaut-Südwestinseln etwa 63 % des Flügels). Aber all diese Merkmale sind gewissermassen zufällig bei den einzelnen Rassen kombiniert, sie lassen keine Beziehungen zu Auslesefaktoren erkennen (nur bei der Grösse ist insofern eine Klimaabhängigkeit deutlich, als die ost-australische *swainsoni* grösser ist als die nächstverwandten

Rassen der östlichen Sunda-Inseln (*xanthogaster*, *flavicollis*). Wichtig ist hier nun die Tatsache, dass die Formen dieser *Ptilinopus*-Gruppe z.T. nun doch so stark differenziert sind, dass sie nicht mehr zu einem einzigen Rassenkreise zusammengefasst werden können. Wohl können *flavicollis* + *ewingi* + *swainsoni* als Rassen von *Pt. xanthogaster* aufgefasst werden und andererseits *huonensis* + *geminus* + *trigeminus* + *quadrigeminus* als Rassen von *coronulatus*, aber die übrigen Formen gelten wegen ihrer weitgehenden Differenzierung wohl mit Recht als eigene Arten. Die richtungslose Variation hat in diesem Falle nicht nur zur Rassen-, sondern auch zur Artbildung geführt. Als ganz paralleles Beispiel könnten etwa die grossen Rassenkreise der Pinselzungpapageien *Trichoglossus ornatus*, des Edelpapageis *Eclectus roratus* oder

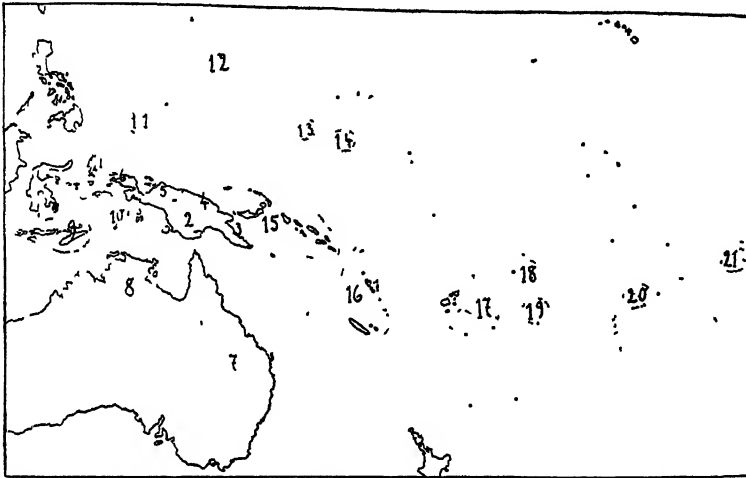


Abb. 2. Verbreitung der Fruchttauben der *Ptilinopus porphyraceus*-Gruppe. 1, *P. monachus*; 2, *P. cor. coronulatus*; 3, *P. c. huonensis*; 4, *P. c. quadrigeminus*; 5, *P. c. geminus*; 6, *P. c. trigeminus*; 7, *P. xanthogaster swainsoni*; 8, *P. x. ewingi*; 9, *P. x. flavicollis*; 10, *P. x. xanthogaster*; 11, *P. pelewensis*; 12, *P. rosei capillus*; 13, *P. ponapensis*; 14, *P. hemsheimi*; 15, *P. richardsi*; 16, *P. greyi*; 17, *P. porphyraceus*; 18, *P. fasciatus*; 19, *P. rarotongensis*; 20, *P. chrysogaster*; 21, *P. tristrani* (Nach Rensch, 1929 a).

der Erddrossel *Pitta cyanura* genannt werden, die ebenfalls über das indoaustroalische Gebiet in verschiedenartig bunt gezeichneten Rassen verteilt sind. Unter den indoaustroalischen Tagfaltern verhalten sich die prächtigen, früher unter dem Namen "Ornithoptera" zusammengefassten *Papilio*-Formen ganz ähnlich: das ♂ von *priamus* (Süd-Mollukken) hat goldgrün und schwarze Flügel, das von *croesus* (Batjan) orangegelb und schwarze, das von *urvilleana* (Neumecklenburg, Salomonen) ultramarinblau und schwarze Flügel usw. Die scharf differenzierten Formen werden z.T. noch als Arten aufgefasst, doch betrachtet sie Jordan (1927) als Glieder eines grossen Rassenkreises (auch in diesem Falle zeigen einige Rassen eine klimaparallele, also nicht richtungslose Verdunkelung auf Inseln mit hoher Niederschlagsmenge).

Wenn es vor allem zahlreiche *Insellformen* sind, bei denen eine solche richtungslose Variation zu neuen Rassen und Arten führte, so wird damit bereits deutlich, dass hier die scharfe *geographische Isolation* von wesentlicher Bedeutung beim

Zustandekommen der bunten Rassenmannigfaltigkeit war. Es ist nun aber natürlich nicht immer nötig, dass dominante Mutanten auf den einzelnen Inseln aufgesprungen sind und ihre Eigenheiten im Laufe der Generationen jeweils über die ganze Inselpopulation ausgebreitet haben. Es genügte in manchen Fällen auch schon, dass eine Population von wenigen Exemplaren ihren Ausgang nahm, welche auf eine Insel verschlagen wurden: entspricht doch der Genbestand einzelner Tiere nie der Totalität der Gene, welche in den zahlreichen Individuen ihrer Herkunftsrasse vorhanden sind. In solchen Fällen ist also *die neu entstehende Inselrasse nicht durch ein Plus an Genen ausgezeichnet, sondern durch ein Minus*. Als Beispiel mögen hier zwei Baumschneckenformen des Rassenkreises *Amphidromus contrarius* genannt sein. Auf der Kleinen Sunda-Insel Timor leben drei geographische Rassen (*contrarius*, *nikiensis*, *hanieli*), die sämtlich sehr stark individuell variieren: die Grundfarbe ist bald weisslich, bald chromgelb, bald bräunlich, die Spiralbänder oberseits der Peripherie sind fortlaufend, unterbrochen, zu Längsflecken verschmolzen oder fehlen völlig. In einer Rasse ist dieser Sunda-Rassenkreis (westwärts bis Sumatra verbreitet) nun ostwärts auch bis zu den kleinen Timorlaut-Inseln vorgedrungen. Hier ist die individuelle Variabilität aber ganz gering (Rasse *columellaris*): alle Schalen sind relativ klein und haben eine chromgelbe Grundfarbe, stets ist ein graurotes Nabelband vorhanden, und stets sind die unterbrochenen Spiralbänder oberhalb der Peripherie zu Längsflecken verschmolzen. Derart gefärbte kleine Exemplare finden sich auch auf der grösseren Insel Timor (von der aus Timorlaut besiedelt wurde), aber nur in einem sehr geringen Prozentsatz, d.h. der Genbestand der Timorlautrasse *columellaris* stellt einen Ausschnitt aus dem Genbestand der variableren Timorform dar. (Im nächsten Kapitel werden noch ähnliche Fälle einer Verminderung des Genbestandes nach dem Rande des Verbreitungsareals hin besprochen werden.)

Nun braucht es sich bei solcher Isolation bestimmter Genbestände natürlich nicht nur um Inseln im geographischen Sinne zu handeln. Alle isolierten Verbreitungsgebiete können in entsprechender Weise derart richtungslos neue Rassen herausbilden. Aber bei grösseren Landkomplexen ist doch die Möglichkeit einer Vermischung beginnender Differenzen meist relativ gross. Zur Erläuterung dieser Verhältnisse seien zwei Landschnecken einander gegenübergestellt, die etwa die gleiche Grösse haben und ähnlich individuell variieren, von denen aber nur die eine in geographische Rassen zerfällt. Es sind das einerseits die europäische Bänderschnecke *Cepaea hortensis* und andererseits *Papuina wiegmanni*, die im wesentlichen nur die Tieflandzone bis 500 m. aufwärts der gebirgigen Insel Neupommern (Bismarck-Archipel), d.h. im wesentlichen nur den Küstensaum bewohnt (vgl. I. Rensch, 1934). Die Schalen haben eine weissliche, gelbliche oder rosa Grundfarbe, einen fast stets schwarzbraunen Mundsaum und oberseits 2 schwarzbraune Bänder von verschiedener Breite und Länge. Bei ihrer Ausbreitung längs der Küste blieb der Genbestand nun aber nicht der gleiche: an der Nordküste finden sich zumeist (bei 92 % der Schalen) nur Varianten, bei denen das untere Band mit dem dunklen Mundsaum verschmolzen ist, an der Südküste herrschen dagegen Schalentypen vor (89 %) bei denen das Ende des unteren Bandes vom Mundsaum durch eine

helle Lucke getrennt ist (Abb. 3). Es konnten deshalb hier zunächst 2 geographische Rassen unterschieden werden: *P. wiegmanni conjuncta* (Nordküste) und *P. w. disjuncta* (Südküste). Im Westteil der Südküste finden sich nun aber noch weitere Varianten: es gibt hier Schalen, bei denen die beiden Bänder des letzten Umganges auf zwei keilförmige Flecken unmittelbar vor dem Mundsaum reduziert sind, dann auch Schalen, bei denen die beiden Bänder miteinander verschmolzen sind, sodass die Oberseite des letzten Umganges fast einheitlich dunkelbraun oder gelbbraun ist und schliesslich auch Schalen, die bei normaler Bänderung einen weisslichen Mundsaum haben. Hier ist also die Färbungsvariabilität erheblich grösser als an



Abb. 3. *Papuana wiegmanni*. Obere Reihe: 3 extreme Varianten von *P. w. wiegmanni* (volle Variationsbreite vom Westteil der Südküste Neu-Pommerns); untere Reihe, links: *P. w. disjuncta* (Ostteil der Südküste); rechts *P. w. conjuncta* (Nordküste). (Nach I. Rensch, 1934.)

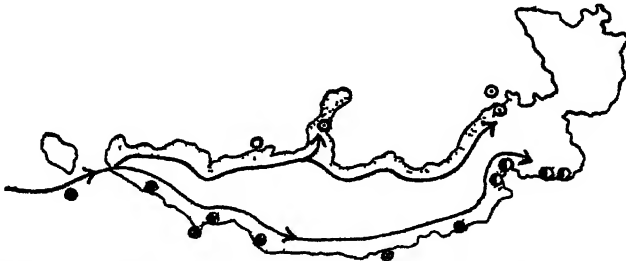


Abb. 4. Verbreitung von *Papuana wiegmanni* auf Neu-Pommern. Schwarze Punkte: Rasse *P. w. wiegmanni* mit voller Variationsbreite; Kreise mit Punkten: Rasse *P. w. conjuncta* mit stark verminderter Variabilität; halbgefüllte Kreise: Rasse *P. w. disjuncta* mit stark verminderter Variabilität. Die Pfeile geben die Ausbreitungsrichtung von Neuguinea her an, die punktierten Flächen das wahrscheinlich besiedelte Gebiet.

der übrigen Südküste und an der Nordküste, und da die normalgebänderten Formen im ganzen nur etwa $\frac{1}{4}$ der Variationsbreite ausmachen, ist es berechtigt, wiederum eine besondere geographische Rasse anzunehmen (*P. w. wiegmanni*). Diese Rasse (Abb. 3) darf wegen ihrer zahlreichen Varianten wohl als Ausgangsform betrachtet werden, und eine solche Annahme wird auch dadurch bekräftigt, dass das Verbreitungsgebiet am nächsten zu Neuguinea gelegen ist, von wo *P. wiegmanni* offenbar eingewandert ist (Abb. 4; auf Neuguinea Rasse *P. wiegmanni kubaryi*), da auch andere verwandte Formen dort leben und da *wiegmanni* die übrigen Inseln des Bismarck-Archipels nicht erreicht hat. Nun ist die Herausbildung der Rassen *disjuncta* und *conjuncta* aber nicht nur durch Genverlust entstanden

(Ausfall von Varianten), sondern es ist an der Nordküste nun auch noch neu ein Färbungstyp aufgetreten, der sich auf der ganzen Insel sonst nicht wiederfindet: es fehlt nämlich einigen Schalen alles Pigment, sodass die Grundfarbe reinweiss und die Bänderung hyalin ist. Solche Varianten finden sich aber nur im Gebiet von *Talassaea* (unter grossen Serien zu etwa 15 %). Es ist also hier eine neue Mutante aufgesprungen, die zur Weiterdifferenzierung des Rassentyps führte.

Eine ähnlich auffällige Färbungsvariabilität wie *P. wiegmanni* zeigen die etwa gleichgrossen europäischen Bänderschnecken der Gattung *Cepaea*. Auch hier finden sich isolierte Populationen, in denen bestimmte Varianten vorherrschen, aber die Kontinuität des Verbreitungsgebietes hat es immer wieder unmöglich gemacht, dass solche Populationen zur Grundlage für die Bildung neuer Rassen wurden, zumindest in Mittel- und Nordeuropa (in Südeuropa Sonderrassen, aber anscheinend durch klimatische Selektion). Vergleichen wir dazu einmal eine Reihe von Populationen von *Cepaea hortensis*, wie sie von mir 1933 zusammengestellt wurden (1933, Tabelle, p. 48). Bei Kuhhorst nördlich Berlins sammelte ich eine Population, die 72.5 % gelbe ungebänderte, 26.4 % fünfbänderige und 1.1 % vierbänderige Schalen enthielt. Wenige hundert Meter davon entfernt wiesen die Schalen noch ein ähnliches Verhältnis auf: 65.3 % ungebänderte, 34.0 % fünfbänderige, 0.7 % vierbänderige. Wiederum einige hundert Meter weiter fand ich dann aber eine bereits ganz anders zusammengesetzte Population mit nur 45.6 % ungebänderten, nur 28.1 % fünfbänderigen, aber 19.3 % vierbänderigen und nun auch 7.0 % dreibänderigen Schalen. Und an einem anderen Fundort nördlich Berlins (Kuhlake bei Spandau) waren die Prozentzahlen wiederum gänzlich andere: hier fanden sich überhaupt keine ungebänderten Stücke, dagegen 98.9 % fünfbänderige und 1.1 % vierbänderige. In einem winzigen Teil des Verbreitungsgebietes der Art zeigen also 4 Populationen eine denkbar verschiedene prozentuale Zusammensetzung der Varianten. Andererseits kehren aber bei weit davon entfernten Populationen manchmal ganz ähnliche Prozentsätze wieder. So fanden sich z.B. bei Hyčice in Böhmen ganz so wie in Kuhlake überhaupt keine ungebänderten Exemplare, aber 97.1 % fünfbänderige und 2.9 % vierbänderige. Weitere Populationen zeigen wiederum ganz andere Zahlenverhältnisse (darunter auch einfarbig rötliche, zweibänderige und andere Varianten). Es handelt sich also um ein *regelloses Vorherrschen bestimmter Varianten in einzelnen Populationen, die infolge gelegentlicher Vermischungen nie zur Ausprägung besonderer geographischer Rassen führen*. Und andere individuell stark variable Tierformen zeigen ganz ähnliche Verhältnisse. Wären solche Formen aber so wie *Papuina wiegmanni* auf einen Küstensaum beschränkt, so hätten sie zweifellos ganz entsprechend auch geographische Rassen ausgebildet.

So zahlreiche weitere Beispiele man nun auch anführen könnte, bei denen eine richtungslose Variation der taxonomischen Hauptmerkmale wahrscheinlich ohne Selektion zur Rassen- oder Artbildung führte (bzw. bei denen eine vielleicht vorhandene Selektion keine Parallelität zur Abänderung verwandter Arten hervorrief), in der gesamten Formenfülle stellen solche Fälle doch nur einen relativ geringen Prozentsatz dar. Dass *einzelne* Merkmale von Rasse zu Rasse richtungslos

abgeändert sind, das dürfte dagegen zumeist der Fall sein, d.h. es sind gewöhnlich richtungslose (biologisch belanglose) Abänderungen mit solchen kombiniert, die in ähnlicher Weise wie bei anderen Arten selektioniert sind (klimatische Parallelität usw.). Bei den Angehörigen der Laufkäfergattung *Carabus* unterliegen z.B. Grösse, Färbung, relative Fühlerlänge, Augenform, u.a. bis zum gewissen Grade parallel einer klimatischen Auslese, die Unterschiede in der Skulptur der Elythren sind dagegen meist regellos (bei *Carabus monilis*-Rassen glattere und schärfere Skulptur, Verschiedenartigkeit der Primär-, Sekundär- und Tertiärintervalle ohne Parallelität zum Klima, vgl. Abb. 1 bei Rensch, 1933).

Wenn solche richtungslose Variation offenbar für den Lebensablauf gewöhnlich mehr oder minder belanglos ist, so kann sie aber doch phylogenetisch wichtig sein, wenn sie *taxonomisch bedeutungsvolle Organe* betrifft. Viele Abänderungen an den komplizierten Genitalorganen von Insekten und Schnecken sind offenbar gänzlich richtungslos, sie können aber bei Häufung der Mutationsschritte zur Copulationsverhinderung und so zu ganz neuen Arten und Gattungen führen. Es seien hier nur die weitgehenden Differenzen genannt, die Jordan (1905) an den Valven von geographischen Schmetterlingsrassen feststellte (Abb. 5): extreme Rassen sind in diesen Fällen schon völlig wie "gute Arten" unterschieden, und sie würden sich vermutlich auch nicht kreuzen, wenn man eine Rasse in dem Gebiet einer geographisch extremen anderen Rasse aussetzen würde. Das wird jedenfalls dadurch wahrscheinlich gemacht, dass in anderen Fällen zwei gute Arten, die unvermischt nebeneinander leben, morphologisch fast identisch sind bis auf die Differenzen in der Genitalarmatur. Auf Korsika-Sardinien lebt z.B. ein Schwalbenschwanz, *Papilio hospiton*, der mit nordafrikanischen Rassen von *P. machaon* verwandt ist und wahrscheinlich von diesen in geologisch junger Vergangenheit abgespalten wurde. Später ist dann auch noch die nordmediterrane Rasse von *P. machaon* auf Korsika und Sardinien eingewandert, die heute unvermischt neben *hospiton* lebt. Beide Formen unterscheiden sich äusserlich nicht sehr von einander, haben aber verschieden gestaltete Valven (vgl. Eller, 1936). Ähnlich leben z.B. in Teilen von Sikkim, Assam, Birma und O. China zwei Falter nebeneinander, *Brahmophyalma wallichii* und *B. hearseyi*, die in Eiern, Raupen, Puppen und Imagines nicht oder nur ganz schwach unterschieden sind, deren Copulationsapparate aber konstante Differenzen aufweisen (vgl. Mell, 1937).

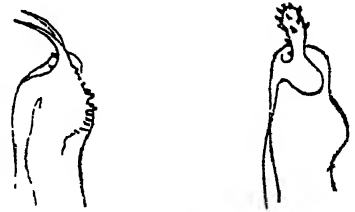


Abb. 5. Oberer Teil der Valve von der nordwestafrikanischen (links) und von der südostafrikanischen Rasse (rechts) des Schmetterlings *Salamis parnassus*. (Nach Jordan, 1905.)

Für ähnliche Fälle unter den Landschnecken sei auf die Gabelung der Glandulae mucosae bei den *Helicigona*-Formen des alpinen und südeuropäischen Gebietes verwiesen. *H. pl. planospira* hat in Mittelitalien bald zwei einheitliche, bald zwei gegabelte Drüsenschläuche (manchmal auch bei gleichen Individuen auf einer Seite gegabelt, auf der anderen ungegabelt (vgl. Hesse, 1930)). Bei anderen *Helicigona*-Formen sind die Drüsenschläuche dagegen stets in gleicher Weise ausgeprägt und gelten als Species-Charakteristikum.

Die Ausprägung richtungslos neugebildeter geographischer Rassen kommt nicht selten auch durch die *Kreuzung zweier Rassen* zustande, wenn die Areale anfänglich völlig getrennt waren und sekundär eine Kontaktzone entsteht. So sind z.B. im Gebiet der Fidschi-Inseln bei dem bunten Würgerassenkreise *Pachycephala pectoralis* dadurch neue Rassen entstanden, dass manche kleinen Inseln von zwei Seiten her mit zwei verschiedenen Rassen besiedelt wurden, die dann eine Bastardform bildeten. So entstand z.B. auf der Insel Koro durch Mischung einer von Norden und Westen kommenden Form, bei der die ♂ eine gelbe Kehle und kein Brustband haben, und einer von Süden kommenden Form, deren ♂ eine weisse Kehle und ein schwarzes Brustband zeigen, eine Zwischenrasse (*koroana*), deren ♂ als neue Kombination eine gelbe Kehle und ein schwarzes Brustband zeigen (vgl. Mayr, 1932). Ähnlich entstand die italienische Rasse des Haussperlings durch Bastardierung des typischen Haussperlings (*Passer dom. domesticus*) mit dem Weidensperling, der heute zwar schon als besondere Art gilt (*P. hispaniolensis*), der aber ein geographischer, bzw. ökologischer Vertreter ist, welcher sich noch überall mit dem Haussperling vermischt, wo er mit ihm im gleichen Biotop zusammentrifft (vgl. Meise, 1936).

Derartige Mischformen brauchen nun aber nicht immer nur eine Kombination der Merkmale der beiden Stammrassen zu zeigen, denn die Vereinigung abweichender Genbestände kann ja bekanntlich phaenotypisch auch "neue" Merkmale zeitigen. Erwähnt sei hier nur das *Luxurieren* von Bastarden, wie ich es in freier Natur im Mischgebiet zweier Schneckenrassen nachweisen konnte. Auf der Kleinen Sunda-Insel Flores gibt es eine Bergrasse *Asperitas trochus badjavensis*, die mit einer Küstenform (*A. t. endeana*) auf halber Berghöhe vermischt ist: hier sind Höhe und Durchmesser der Schalen durchschnittlich deutlich erhöht (vgl. Rensch, 1932 b, pp. 14-16 u. Taf. 1).

Rasse	Hohe in mm	durchschn.	Durchm. in mm	durchschn.
<i>badjavensis</i>	24-32	28	28-34	31
Mischrasse	22-39	31	28-42	35
<i>endeana</i>	24-29	26.5	30-35.5	32

Nach allem, was bisher an Kreuzungen in freier Natur bekannt ist, hat es den Anschein, dass auf diesem Wege nur neue geographische Rassen zustande kommen. Bei den Bastardierungen "guter" Arten, die fast in allen Tiergruppen gelegentlich auftreten, ist wohl stets die Fertilität oder Vitalität der Nachkommen etwas vermindert, sodass solche Mischformen nicht zu neuen "guten" Arten führen. Wenn Ghigi (vgl. z.B. 1931) bei Hühnervögeln die Entstehung von Arten durch Bastardierung experimentell erwiesen zu haben glaubt, so ist dem entgegenzuhalten, dass die Kreuzungspartner stets nur geographische Vertreter, also nie gute Arten waren (man vgl. auch Stresemanns frühere Einwände (1919) gegen Artbildung durch Kreuzung). Doch darf man wegen der Fertilität mancher Artbastarde, wie sie z.B. in Whitmans Taubenkreuzungen (1919) durch mehrere Generationen nachgewiesen wurde (Vitalität nicht exakt geprüft), eine gelegentliche Neubildung von Arten vielleicht nicht völlig ausschliessen. Jedenfalls handelt es sich dann aber um ganz

seltene Ausnahmen, denn normalerweise sind *schon bei extremen geographischen Rassen eines Rassenkreises die physiologischen Konstanten so weit verschieden, dass eine Fertilitäts- oder Vitalitätsminderung der Bastarde eintritt* (erinnert sei nur an die zahlreichen Intersexe, die in Goldschmidts Zuchten bei Kreuzungen von fernerstehenden *Lymantria*-Rassen auftraten). Bei Pflanzen liegen die Verhältnisse ganz anders. Hier ist der Chemismus von verwandten Arten und manchmal auch von Gattungen noch so wenig unterschieden, dass Bastardierungen zu einer lebenskräftigen Nachkommenschaft führen. Daher ist hier eine richtungslose Neubildung nicht nur von Rassen, sondern auch von Arten durch Bastardierung möglich (infolgedessen auch die taxonomischen Verhältnisse z.T. anders als beim Tierreich: vgl. Rensch, 1934 a, Kap. 16 u. 1938 b).

III. GERICHTETE ARTBILDUNG OHNE ERKENNBARE SELEKTION

Es ist eine den Systematikern seit langem geläufige Erscheinung, dass *bei verwandten Arten oder verwandten Gattungen vielfach die Variabilität erblicher Merkmale eine gewisse Parallelität zeigt*. So variieren z.B. die Schalen unserer Bänderschnecken *Cepaea hortensis*, *C. nemorensis* u. *C. vindobonensis* in der Weise, dass einzelne Bänder ausfallen oder dass die Bänder z.T. oder sämtlich miteinander verschmolzen sind (die artunterscheidenden Merkmale sind andere: Färbung der Mündung, Rippung, anatomische Sonderheiten). Es ist das zunächst nur ein Ausdruck für die physiologische Ähnlichkeit verwandter Formen, bzw. für die teilweise Identität der zugrundeliegenden Genstruktur. *Gelegentlich springen aber parallele Mutanten auch dann noch auf, wenn die Differenzierung bereits soweit fortgeschritten ist, dass die Variationsbreiten nicht mehr ähnlich sind*. Es mag das an einer Landschneckengruppe des Mittelmeergebietes aus der Familie der Heliciden verdeutlicht werden. Auf Sizilien gibt es eine grosse Zahl von *Murella*-Formen, die im Schalenbau ausserordentlich verschieden sind. Manche Formen sind stumpfkegelig oder kugelig mit glatter Oberfläche, andere sind flach, scharf gekielt und gerippt oder gerunzelt. Wie ich durch Kreuzungsversuche und durch Studium der Verbreitung zeigen konnte (Rensch, 1937), handelt es sich aber trotz dieser weitgehenden Unterschiede nur um geographische Rassen eines Rassenkreises (alle Formen ersetzen einander geographisch; Zwischenformen in den Grenzgebieten, die den experimentell erzeugten Bastarden ähnlich sind; im übrigen weitgehende anatomische Übereinstimmung). Da nun die Areale der flachen, gekielten Formen an mehreren (ursprünglich zumindest zwei) Stellen zwischen die Areale ungekielter Formen eingeschaltet sind, müssen wir annehmen, dass sie parallel zweimal aus ungekielten Formen (welche die phylogenetisch älteren sein werden) entwickelt worden sind.

Eine solche Auffassung wird nun bestärkt durch die wichtige Tatsache, dass auch bei anderen Rassenkreisen der gleichen Verwandtschaftsgruppe solche flachen, gekielten und gerippten Rassen aus rundlichen kegeligen Formen abgespalten wurden (Abb. 6). So findet sich auf Sardinien ganz ähnlich wie auf Sizilien der kugelig-kegelige glattschalige *Tyrrheniberus villicus* und der flache, gekielte und gerippte *sardonius* als einander ersetzende geographische Vertreter mit etwa

gleichen (aber von *Murella muralis* stark abweichenden) anatomischen Verhältnissen. Die Form *sardonius* ist dabei sogar in der Schalenform den gekielten, flachen *Murella*-Rassen zum Verwechseln ähnlich. Das Gleiche wiederholt sich in Marokko, wo die rundlichen oder kegeligen Schalen des *Rossmassleria*-Rassenkreises (Anatomie von der von *Murella* und *Tyrrheniberus* abweichend) wiederum eine flache gekielte und gerippte Rasse (*subscabriuscula*) gebildet haben. Und ganz entsprechend verhalten sich wiederum in Südostspanien *Iberus gualterianus alonensis* (rundlich) und *I. gualt. gualterianus* (gekielt und gerippt), in Tripolis *Levantina g. gyrostoma* (rundlich) und sein geographischer Vertreter *L. g. leachi* (gekielt und gerippt) und in der Libyschen Wüste *Eremina hass. hasselquisti* (rundlich) und *E. h. zitteli* (gekielt) (photographische Abbildungen aller Formen in Rensch, 1937, PP. 574-575).



Abb. 6. Drei Rassenpaare mediterraner Trockenschnecken zur Veranschaulichung paralleler geographischer Variation infolge "Pluripotenz". 1, *Murella mur. muralis* (oben) und *M. m. segestana* (unten); 2, *Tyrrheniberus vill. villica* (oben) und *T. v. sardonius* (unten); 3, *Rossmassleria (sub.) boettgeri* (oben) und *R. (sub.) subscabriuscula* (unten). Die 3 Gattungen sind anatomisch unterschieden. (Nach Rensch, 1937.)

Es konnten nun bisher keine Umweltfaktoren aufgefunden werden, die für die Auslese flacher, gekielter Varianten, d.h. für die Herausbildung der entsprechenden geographischen Rassen verantwortlich gemacht werden könnten, denn sie finden sich sowohl an der Küste als auch weiter im Binnenlande (nur die ausgesprochen rundlichen Formen, wie *Murella muralis sicana* auf Sizilien, sind wohl auf die feuchtere Luft der Küste angewiesen). Es liegt also in der besprochenen Verwandtschaftsgruppe mediterraner Heliciden eine generelle "Pluripotenz" (im Sinne Haeckers) vor, solche flachen, gekielten und gerippten Mutanten aufspringen zu lassen. Das bedeutet also: *die richtungslose Variabilität ist hier durch bestimmte Mutationspotenzen eingeeengt, sodass in den verschiedensten Rassenkreisen parallel ähnliche Rassen ohne Einwirkung einer Selektion entstehen*. Ähnliche Fälle einer gerichteten Rassenbildung liegen wahrscheinlich mehrfach vor. Gelegentlich sind solche "Mutationspotenzen" wohl auch dadurch bedingt, dass eine *kryptomere Musterung* vorliegt, welche nur eine bestimmte Ausprägung des Phaenotypus zulässt. In den Untersuchungen der Vogt'schen Schule über "eunomische Reihen" finden sich Beispiele für eine solche gerichtete Variabilität (untersucht wurden vor allem Zeichnungen von Hummeln und Coccinelliden (vgl. z.B. Vogt (1911) und Zarapkin (1930)).

Im II. Kapitel haben wir Beispiele kennen gelernt (*Amphidromus*, *Papuina*), bei

denen eine Rassenbildung durch Verminderung des Genbestandes dadurch eingetreten ist, dass relativ wenige Exemplare mit unvollständigem Genbestand eine Population begründeten. Es kommt nun auch gelegentlich vor, dass bei der Ausbreitung einer Tierart eine solche *Allelminderung ohne erkennbare Selektion derart fortlaufend eintritt, dass die Rassenbildung gerichtet erscheint*. Als Beispiel möchte ich hier die sukzessive Abnahme der Variabilität bei der Süßwasserschnecke *Melania tuberculata* (Lam.) von Ost nach West anführen. Es gibt hier Varianten mit völlig glatter Schale, solche mit eingeschnittenen Spirallinien nur auf der Unterseite der Umgänge, mit eingeschnittenen Spirallinien auf den ganzen Umgängen und schliesslich solche mit erhabenen Spiralleisten. Im Herkunftsgebiet der Art und der Gattung, in der malayisch-papuasischen Inselwelt, finden sich alle 4 Typen in wechselnder Häufigkeit, in Hinterindien fehlen bereits der glattschalige und der wenig spiralige Typ, während der Typ mit aufgesetzten Spiralleisten in 83 % der Fälle auftritt. In Vorderindien und in Afrika ist dieser Typ dann aber mit 98–99 % vorherrschend, d.h. die Populationen machen hier einen sehr einheitlichen Eindruck.—Als entsprechende Fälle könnten die Abnahme der Färbungsvariabilität der Bänderschnecke *Cepaea hortensis* von Mitteleuropa nach Nordskandinavien hin oder die Abnahme der Variabilität von *Helix pomatia* vom Balkan nach Deutschland hin genannt werden.

Reinig (1937) bezeichnete einen solchen Ausfall von Allelen ohne Selektion als "Elimination". Er ist der Meinung, dass ein grosser Teil der postglazialen Rassenbildungen auf diese Weise zustande gekommen ist. Das dürfte indess wohl keineswegs zutreffen, und Reinig vermag auch die Wirksamkeit einer Selektion gewissermassen nur theoretisch, auf Grund unbewiesener bzw. unbeweisbarer Voraussetzungen, auszuschliessen. Wir werden im nächsten Kapitel noch genauer darauf zu sprechen kommen.

Ein anderer Typ gerichteter Rassen- oder Artbildung liegt dann vor, wenn innerhalb einer Ahnenreihe die Folge der zur Weiterentwicklung führenden Mutationschritte in gleicher Richtung liegt, d.h. wenn eine *orthogenetische Entwicklung* vorliegt. Derartige Fälle, die früher mehrfach zur Annahme einer inneren phylogenetischen Gestaltungstendenz führten, sind heute z.T. bereits so weit analysiert worden, dass eine kausale Deutung zumindest wahrscheinlich gemacht werden konnte.

Zunächst sei darauf hingewiesen, dass das Auftreten "gerichteter Mutationen", d.h. einer abgestuften Mutantenfolge (Änderung in Allelenreihen) wie sie Jollos (1930, 1932) durch Behandlung mehrerer Generationen von *Drosophila melanogaster* mit Temperaturschocks erhielt, bei den sorgfältigen Nachprüfungen von Plough und Ives (1932, 1935) nicht bestätigt werden konnte. Von der Diskussion einer experimentellen Herstellung orthogenetischer Reihen kann hier deshalb vorläufig abgesehen werden.

Weiterhin wollen wir die Fälle beiseite lassen, bei denen eine gerichtete Weiterentwicklung wahrscheinlich durch eine gerichtete Selektion ("Orthoselektion") zustandekommt. Das ist offenbar der Fall bei der bekannten Grössenzunahme innerhalb der Stammesreihen (Cope-Dépérétsche Regel): bei den Jungen eines

Tieres sind fast immer die grösseren Varianten die erhaltungsfähigeren (Castle, 1932; vgl. Kap. VI). Und auch die Ketten geographischer Rassen, die in geographischer Folge die Steigerung bestimmter Merkmale zeigen (Grösse, Farbe, Proportion u.a.) dürften zumeist durch Selektion zustande kommen (klimatische Auslese, Kap. IV).

Doch bleibt immer noch eine nicht geringe Zahl von Entwicklungsreihen über, bei denen die Orthogenese auf "inneren" Tendenzen beruhen muss. Genannt seien nur die zunehmende Ausprägung der frontalen Hörner der Titanotherien und der Kopf- und Thoraxfortsätze bei Käfern der Gattung *Golofa*. Hier haben nun die Erfahrungen über heterogones Wachstum, wie sie vor allem in Huxleys hervorragenden und noch viel zu wenig beachteten Untersuchungen (1932) gewonnen wurden, eine überraschend einfache und völlig ausreichende Erklärungsmöglichkeit geschaffen. Wir werden im V. Kapitel näher darauf eingehen.

IV. GERICHTETE AUSLESE BEI DER ARTBILDUNG

Wie im II. und III. Kapitel ausgeführt wurde, ist es bei der gelegentlich feststellbaren Verringerung der Variabilität nach dem Rande der Verbreitung zu zumeist noch nicht zu entscheiden, ob hier nur ein Genverlust durch den Vorgang der Ausbreitung selbst (Elimination) vorliegt, oder ob es sich um die Folgen einer schärferen Selektion handelt (was zumeist wahrscheinlicher ist). In anderen Fällen ist nun aber eine Auslese in bestimmter Richtung unverkennbar. Wenn sehr viele Insekten oder tropische Baumschnecken, die auf grünen Blättern entsprechend grüne Färbungen aufweisen (unter Schnecken der Kleinen Sunda-Inseln z.B. am eindeutigsten entwickelt bei *Asperitas everetti*, *A. bimaënsis halata* und *A. b. cochlostyloides*, *Amphidromus furcillatus*, *Leptopoma vitreum*, *Sulfurina biconica*), so liegt hier offenbar eine gleichgerichtete Auslese zugrunde. Und entsprechende Fälle liegen bei der Färbungsanpassung an Sand, Schnee, Rinde usw. vor. Derartige Beispiele wurden aber bereits so viel diskutiert, dass hier davon abgesehen werden kann.

Wichtiger sind die *Fälle einer klimaparallelen Merkmalsausprägung*, deren generelles Auftreten im wesentlichen erst in den letzten beiden Jahrzehnten erkannt bzw. genauer studiert wurde. Dass es sich dabei fast ausnahmslos um *erbliche* Charaktere handelt, konnte in einigen Fällen erwiesen, in anderen wahrscheinlich gemacht werden. Da hier die Abänderungen an geographischen Rassen vieler Rassenkreise (Arten) mehr oder minder unabhängig von den speziellen Lebensgewohnheiten auftreten (also z.B. sowohl bei Raubtieren wie bei Beutetieren, bei fliegenden, wie bei nicht fliegenden Tieren), so müssen die klimatischen Faktoren, denen die Merkmale parallel laufen, die Auslese bewirkt haben. Die Parallelität ist dabei eine so weitgehende, dass bei homöothermen Tieren bereits generelle biologische *Klima-regeln* formuliert werden konnten. Ich habe diese Regeln verschiedentlich ausführlicher begründet und diskutiert (vgl. besonders Rensch, 1936, 1938 a), sodass ich mich hier auf eine kurze Zusammenfassung beschränken kann, wobei lediglich einige erst in letzter Zeit genauer untersuchte Einzelheiten besprochen werden sollen.

(1) BERGMANNSCHE REGEL. Innerhalb eines Warmblüter-Rassenkreises sind im allgemeinen die Rassen in kühleren Gebieten grösser als in wärmeren Zonen. (Von Bergmann 1847 für Arten einer Gattung aufgestellt, von mir 1924 auf Rassen von Rassenkreisen (Arten) beschränkt und durch prozentuale Berechnung von Ausnahmen als gültig erwiesen.) Die Grössendifferenzen beruhen dabei auf verschiedener Zellzahl bei gleicher Zellgrösse (Rensch, 1929 b). Nach Svilhas Messungen an wachsenden *Peromyscus*-Formen (1935) kann weiterhin gefolgert werden, dass bei grossen Rassen lediglich das Tempo der Zellteilungen beschleunigt ist.

Reinig (1938) versuchte nun kürzlich nachzuweisen, dass die Bergmannsche Regel unzutreffend sei. Seiner Meinung nach läuft die Grössenabnahme nicht einer Temperaturzunahme parallel, sondern es findet nur eine Verringerung der Körpermasse infolge einer Elimination von Polymeriefaktoren vom Ausbreitungszentrum zur Peripherie hin statt. Wie ich in einer ausführlichen Kritik (1938 a) feststellen konnte, gründet sich aber seine Beweisführung auf z.T. sehr hypothetische Annahmen bezüglich der mutmasslichen postglazialen Ausbreitungswege, der Ausbreitung durch Einzelwanderung und der Elimination von Allelen. Vor allem setzt Reinig voraus, dass Mutation und Selektion bei der postglazialen Ausbreitung praktisch wirkungslos war, was keineswegs bestätigt werden kann. Es genügt auch nicht, wenn er einzelne Beispiele als Beweise anführt, denn es gibt ja einen bestimmten Prozentsatz von Ausnahmen für die Bergmannsche Regel (s.o.). Aber selbst diese Einzel-Beispiele entsprechen sogar zumeist noch der Bergmannschen Regel, wenn man extreme Rassen vergleicht. Die angenommenen postglazialen Ausbreitungswege stimmen zudem z.T. auch mit den pleistocänen Fossilfunden nicht überein. Schliesslich konnte ich noch zeigen, dass auch Ausnahmen der Bergmannschen Regel nicht generell durch den von Reinig angenommenen Allelverlust gedeutet werden können. So muss diese Eliminationshypothese als völlig unbewiesen betrachtet werden.

In der gleichen kritischen Besprechung (1938) konnte ich nun nachweisen, dass die Bergmannsche Regel aber noch in bestimmter Weise zu erweitern ist. Durch Huxleys hervorragende Untersuchungen über heterogones Wachstum der Organe (1932) angeregt, prüfte ich nach, in welchem Ausmasse die exponierten Körperteile der homöothermen Tiere, wie Schwänze, Ohren, Schnäbel und Füsse in ihren relativen Massen bei verschiedenen grossen Individuen einer Population verändert sind. Es ergab sich dabei, dass eine *Zunahme der Körpergrösse fast generell mit einer Abnahme der relativen Länge exponierter Organe verbunden* ist, wie dies auch Zimmermann (1937) schon einmal an einem Einzelbeispiel (*Clethrionomys glareolus*) gezeigt hatte. Das bedeutet also, dass Schwänze, Ohren, Schnäbel und Füsse zumeist ein negativ-heterogones Wachstum gegenüber dem Kopf-Rumpfgebiet aufweisen (Abb. 7). Die Bergmannsche Regel kann infolgedessen entsprechend erweitert werden: *erbliche Warmblüterrassen kühlerer Gebiete haben gegenüber den Rassen aus wärmeren Gebieten bei unveränderter Zellgrösse ein beschleunigtes Körperwachstum, das zu einer bedeutenderen Körpergrösse aber geringeren relativen Länge der exponierten Körperteile führt.*

Damit sind nun auch die Erklärungsmöglichkeiten für das Zustandekommen der Bergmannschen Regel erweitert. Wie schon Bergmann selbst mit Recht hervorgehoben hatte, ist es für einen Warmblüter vorteilhaft, im kühleren Gebiet grösser zu sein, weil die Auskühlung des Körpers damit verringert wird, denn die Oberfläche wächst ja nur im Quadrat, der Inhalt aber im Kubus. Es würden also im kühleren Gebiet derartige grössere Varianten von der Auslese begünstigt sein. Nun kann aber die Selektion weiterhin auch durch die relativen Differenzen der exponierten Körperteile verschärft werden, denn diese Organe sind ja von grosser Bedeutung für die Temperaturregulierung. Vor allem wird es damit auch besser verständlich, dass nicht nur eine Auslese grösserer Varianten im kühleren Klima stattfindet, sondern auch eine Auslese kleinerer Varianten im wärmeren Klima (z.B. bei Ausbreitung paläarktischer Tiere wie *Parus major* in die Tropen hinein): hier ist offenbar weniger die Verminderung der Körpergrösse wichtig als vielmehr die damit gekoppelte Vergrösserung in der relativen Länge von Schwänzen, Ohren und Füssen, die eine erhöhte Abkühlung bewirkt. So fügen sich also jetzt auch solche Fälle einer Selektionserklärung leichter ein, die bisher besondere Schwierigkeiten bereiteten (auf die ich selbst mehrfach, besonders 1933 hingewiesen hatte).

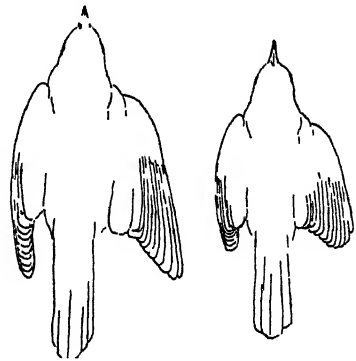


Abb. 7. Zwei Rassen des Drossenrohrsängers. Links: *Acrocephalus ar. arundinaceus* (Mitteleuropa); rechts: *A. ar. meyeri* (Bismarck-Archipel). Zur Veranschaulichung der Bergmannschen, Allenschen und der Flügelschnittregel.

Schliesslich sei auch noch auf einen bisher nicht berücksichtigten Gesichtspunkt hingewiesen. Da die Zellgrösse bei grossen und kleinen Rassen des gleichen Rassenkreises die gleiche ist, haben die ersteren auch *relativ* mehr rote Blutkörperchen, d.h. die resorbierende Oberfläche ist nicht nur absolut, sondern auch relativ vergrössert. Eine grössere Rasse begegnet also dem kühleren Klim durch drei miteinander gekoppelte Vorteile: relativ geringere Oberfläche des Körpers, relativ geringe Grösse der zur Auskühlung wichtigen exponierten Körperteile und relativ erhöhten Stoffwechsel. Dadurch wird es verständlich, dass auch verhältnismässig geringfügige Differenzen der durchschnittlichen Körpergrösse schon für eine klimatische Auslese Bedeutung haben.

(2) ALLENSCHE REGEL. *Innerhalb eines Warmblüterrassenkreises haben im allgemeinen die Rassen kühlerer Gebiete relativ kürzere Schwänze, Ohren, Schnäbel und Füsse als die Rassen wärmerer Zonen.* (Zuerst von Allen 1877 für Arten einer Gattung oder Familie aufgestellt, später an Beispielen mehrfach bestätigt, von mir selbst 1929 auf Rassen eines Rassenkreises beschränkt und durch prozentuale Berechnung von Ausnahmen erwiesen, vgl. besonders Rensch, 1936.)

Die oben besprochene Feststellung, dass die Ausprägung der relativen Länge exponierter Körperteile mit der Körpergrösse gekoppelt ist, lässt zunächst die Beibehaltung einer gesonderten Allenschen Regel als überflüssig erscheinen. Sie muss aber doch aufrecht erhalten bleiben, weil sie auch zutrifft, wenn die Berg-

mannsche Regel versagt. Wie ich kürzlich (1938a) an einer Reihe von Beispielen zeigen konnte, haben Rassen wärmerer Gebiete oft auch dann relativ längere Schwänze, Ohren oder Schnäbel, wenn sie ebenso gross oder gar grösser sind als Rassen kühlerer Länder.

Die Regel kann durch klimatische Auslese entsprechender Varianten gedeutet werden, weil die exponierten Körperteile für die Temperaturregulierung wichtig sind. Schwierigkeiten bereiten hier nur die Differenzen relativer Schnabellänge bei Vögeln, weil diese fast nur aus Horn und Knochen bestehenden Gebilde kaum zur Abkühlung der Körpertemperatur beitragen können. Soweit in wärmeren Gebieten relative Vergrösserung des Schnabels und absolute Verringerung des Gesamtkörpers gekoppelt sind (wie das zumeist der Fall ist) mag die Selektion an letzterem Merkmal angegriffen haben und bei Versagen der Bergmannschen Regel kann schliesslich auch die mit der Schnabelvergrösserung gekoppelte relative Tarsusvergrösserung für die Selektion ausschlaggebend gewesen sein (Beispiele bei Rensch, 1938a, Tabellen 5–6).

Da es zu weit führen würde, auch die übrigen Klimaregeln genauer zu besprechen, und da hier auch keine neuen Gesichtspunkte zu erörtern sind, mag es genügen, auf meine genannten, einschlägigen Arbeiten (besonders von 1936 und 1934) zu verweisen und die Regeln hier nur zu nennen.

(3) FLÜGELSCHNITTREGEL. *Innerhalb von Vogelrassenkreisen (Arten) sind die Rassen kühlerer Gebiete im allgemeinen durch relativ schmalere und spitzere, d.h. flugmechanisch wirksamere Flügel von den Rassen wärmerer Zonen unterschieden.* Diese Änderung des Flügelschnittes kommt zustande durch relative Verkürzung der 1. Schwinge, der Armschwingen und der hinter der längsten Schwinge gelegenen Handschwingen und durch Verlängerung der nach dem Flügelrand zu gelegenen flugmechanisch besonders wirksamen Schwingen. (Einzelne Beispiele bei Averill, 1925, und Kleinschmidt, 1925, 1933; als Regel formuliert und an grösserem Materiale durch prozentuale Berechnung von Ausnahmen erwiesen: Rensch, 1938c.)

(4) GLOGERSCHE REGEL. *Innerhalb eines Warmblüter-Rassenkreises weisen die in wärmeren und feuchteren Gebieten beheimateten Rassen eine stärkere Melaninpigmentierung auf als die Rassen kühlerer und trockener Gebiete. In trockenwarmen Gebieten lebende Rassen haben vorwiegend gelbe oder rotbraune Melanine. In kälteren Gebieten herrschen schwarzbraune Melanine vor, die in arktischen Gebieten reduziert werden. Vögel trockenwarmer Gebiete haben blassere Lipochrome.* (Grundlagen der Regel bei Gloger, 1833, Allen, 1877, später hauptsächlich ausgearbeitet von Görnitz, 1923; vgl. Rensch, 1936).

(5) HAARREGEL. *Innerhalb eines Säugetier-Rassenkreises haben die in wärmeren Gebieten beheimateten Rassen im allgemeinen relativ kürzere und relativ breitere Grannenhaare und weniger Wollhaare als die Rassen kühlerer Länder* (Rensch, 1936).

(6) EIREGEL. *Innerhalb eines Vogelrassenkreises legen im allgemeinen die Rassen kühlerer Gebiete mehr Eier pro Gelege als die Rassen wärmerer Gebiete.* Der Unterschied wird, soweit bisher feststellbar, nicht durch zusätzliche Gelege in wärmeren Gebieten kompensiert (Grundlagen bei Prinz M. zu Wied, 1830, Schomburgk,

1848, Hesse, 1922, 1924, Snethlage, 1928; Formulierung und prozentuale Ausnahmeberechnung: Rensch, 1934c).

Parallel damit läuft wahrscheinlich eine entsprechende *Wurfgrößenregel für Säugetiere*: Zahlenmaterial bei Rensch, 1936.

(7) ZUGREGEL. *Bei grösseren Zugvogel-Rassenkreisen, die von den gemässigten Zonen bis in die Tropen hineinreichen, besitzen die tropischen Rassen keine Zuginstinkte* (Rensch, 1936).

Es dürfte bei homöothermen Tieren noch eine Anzahl weiterer Regeln klimatischer Merkmalsparallelität feststellbar sein. Wahrscheinlich ist dies vor allem für die relative Verkleinerung von Magen und Darm bei Vogelrassen wärmerer Gebiete (Zahlenmaterial für 2 Arten bei Rensch, 1930).

Bei *poikilothermen Tieren* verhält sich die *Körpergrösse* dem Klima gegenüber prinzipiell anders als bei homöothermen Formen. Mell (1929) untersuchte die Zahl der Ventral- und Subcaudalschuppen bei chinesischen Schlangentrassen, die zugleich einen Masstab für die Körpergrösse darstellen. Er kommt dabei zu folgender Regel: "Reptilien und wahrscheinlich poikilotherme Lebewesen überhaupt erreichen ihre Maxima im Gebiet ihrer grössten Häufigkeit, in der optimalen Region ihres Arealis."

Im gleichen Jahre (1929) konnte ich selbst bei einer Zusammenstellung von Odhner ermittelter Masszahlen (1912, 1915) für arktische marine Mollusken eine Grössenzunahme nach Norden hin ermitteln, wobei typischerweise die Mehrzahl der 5 Ausnahmen (bei 24 vergleichbaren Formen) auch in gemässigten Teilen des Atlantik (z.B. Portugal) vorkommt, also in der Arktis nicht mehr ihre normalen bzw. optimalen Lebensbedingungen findet.

Später untersuchte mein Schüler Shih (1937) auf meine Anregung hin an umfassenden Material eine grössere Zahl mariner Mollusken und fand dabei ebenfalls, dass eine Grössenabnahme vom Optimum zum Pessimum hin (hinsichtlich Temperatur und Salzgehalt) stattfindet. Dagegen ist das relative Schalengewicht allgemein innerhalb der gleichen Art in kälteren Meeren geringer als in wärmeren.

Diese Ergebnisse entsprechen auch im allgemeinen den Regeln, die ich bereits 1932 für Landmollusken (15 europäische Arten in 3430 Exemplaren) aufstellen konnte: "*Jede Art zeigt eine Zunahme der Schalengrösse bis zu einem bestimmten Optimum in der Temperatur und der durchschnittlichen jährlichen Niederschlagsmenge hin und eine Abnahme, sobald das Optimum überschritten ist.*... Die nicht xerophilen Formen werden stärker von der Feuchtigkeit beeinflusst, die xerophilen Formen dagegen mehr von der Temperatur, d.h. diese werden oftmals im wärmeren Gebiet auch dann noch grösser, wenn es erheblich trockener ist. Es gibt auch Arten, deren Grössenoptimum in ausgesprochen kühlen Gebieten liegt (z.B. Nordskandinavien) "Zumeist sind diese Grössenänderungen auf eine Zunahme der Umgangsanzahl zurückzuführen, wobei sich die Proportionen gewöhnlich ändern: flache Schalen werden automatisch durch Vermehrung der Umgänge relativ flacher und getürmte Schalen automatisch schlanker." "Die relative Schalendicke ist abhängig von der Stärke der Besonnung und von Kalkgehalt des Untergrundes."

Weitere Regeln fand mein Schüler H. Knipper (*Arch. f. Naturgesch.* 1939 im

Druck) bei der Untersuchung balkanischer Heliciden. Erwähnt sei hier vor allem die Zunahme der *relativen Mündungsgrösse* im feuchteren Klima.

Auch bei anderen poikilothermen Tieren, besonders bei Insekten, liegen schon so viele Einzelbeispiele für klimaabhängige Grössenabstufungen vor, dass ähnliche Regeln in Zukunft leicht aufgestellt werden können. Leider können wir aber zumeist noch nicht beurteilen, ob es sich hier um erbliche Differenzen oder nur um Modifikationen handelt. Kreuzungsversuche mit Rassen einer sizilianischen Trockenschnecke (*Murella muralis*) liessen jedenfalls erkennen, dass Grösse und Schalendicke leicht modifikatorisch zu beeinflussen sind (Rensch, 1937).

Reinig (1938) möchte auch bei poikilothermen Tieren diese Grössenabstufung nicht auf klimatische Selektion, sondern auf Elimination von Allelen vom Ausbreitungszentrum zur Peripherie hin erklären. Er übersieht dabei aber, dass gelegentlich die grössten Formen an der Peripherie der Verbreitung leben (z.B. europäische Bänderschnecke *Cepaea nemoralis* im südlichsten Teile der Verbreitung, in Italien, am grössten; europäische Schnecken *Euconulus*, *Phenacolinax*, *Columella* in N. Skandinavien am grössten). Im übrigen wird zumeist Ausbreitungszentrum und Grössenoptimum zusammenfallen.

Auch für die *relative Länge exponierter Organe* sind bei den *Poikilothermen* schon Ansätze für Klimaregeln vorhanden. So fand Hellmich (1934) bei 2 chilenischen Eidechsen-Rassenkreisen der Gattung *Liolaemus* parallel eine relative Verkürzung des Schwanzes und der Extremitäten von Norden nach Süden. Diese Gestaltänderung wird in Zusammenhang mit der Lebensweise gebracht. Die Parallelität zur Allenschen Regel bei Homöothermen lässt allerdings vermuten, dass hier primär eine Klimaparallelität selektiv Veränderungen bewirkte, die sich sekundär dann auch in der Wahl des Biotops äusserte. Leider ist das Zahlenmaterial zu gering um nachzuprüfen, ob es sich bei Schwanz und Extremitäten nur um ein negativ heterogones Wachstum gegenüber dem Kopf-Rumpfwachstum handelt, ob also die Proportionsänderung durch die Grössenänderung automatisch bedingt ist (s.o.). Besser können wir diese Verhältnisse bei den von Krumbiegel (1936 a, b) eingehend analysierten *Carabus*-Rassen beurteilen. Auch hier konnte durch ausserordentlich umfangreiche Messungen eine zunehmende relative Verkürzung von Fühlern, Tastern und Beinen im kühleren Klima nachgewiesen werden. Diese relativen Differenzen zeigten sich aber auch beim Vergleich gleichgrosser Individuen aus verschiedenen Klimaten: es handelt sich also hier offenbar nicht nur um ein mit der Körpergrösse gekoppeltes negativ heterogones Wachstum. Bei den Caraben können wir zudem schon fast von einer "Regel" sprechen, denn es wurde bei 8 in dieser Weise untersuchten Rassenkreisen bisher nur eine Ausnahme gefunden. Wichtig ist es, dass die längeren Fühler auch relativ viel mehr Geruchskegel besitzen, dass sie also funktionell dem mehr räuberischen Leben der Rassen in wärmeren Ländern entsprechen.

Die *Färbung poikilothermer Tiere* läuft oft ähnlich wie bei den homöothermen Tieren den Klimafaktoren parallel. Allgemein verbreitet ist eine Zunahme der Melanine in feuchteren Gebieten und ein Ersatz der schwarzbraunen durch rötliche und gelbliche Melanine in trockenwarmen Gebieten (vgl. z.B. von neueren Arbeiten

Hellmich, 1934 für Eidechsen, Vogt, 1909, 1911, Krüger, 1931 und Reinig, 1930 für Hummeln, Zimmerman, 1931 für Vespiden, Th. Dobshansky, 1927, 1933 für Coccinelliden, Netolitzky, 1931 für verschiedene Käfer usw.). Da ausser Wärme und Feuchtigkeit auch noch verschiedene andere Faktoren auf die Melaninpigmentierung einwirken, sind die Verhältnisse aber keineswegs ganz einheitlich und eine Deutung durch Selektion bestimmter Mutanten ist schon wegen der sehr verschiedenartigen genetischen Grundlage der Melaninpigmentierung schwierig. Es würde hier zu weit führen, die umfangreiche einschlägige Literatur kritisch zu besprechen, und es mag die Feststellung genügen, dass es sich auch hier um eine Parallelität mit klimatischen Faktoren handelt. In einer Zusammenstellung über Melanismus und Albinismus möchte Reinig (1937) freilich im wesentlichen nur die Isolation von Populationen für die Entstehung eines erblichen Melanismus verantwortlich machen, er vermag diese Ansicht aber meines Erachtens nicht ausreichend zu stützen. Sie trifft vielleicht für einen Teil der Inselelismen zu, wie dies schon von anderer Seite betont wurde, aber z.B. wohl sicher nicht für Moorgebiete, denn hier müssten sonst ja auch andere rezessive Mutanten gehäuft sein. Zudem treten Verdunkelungen in feuchteren Gebieten doch auch bei kontinuierlicher Verbreitung auf.

Schliesslich sei nur noch auf die bekannte Abhängigkeit der *Wirbel- und Flossenstrahlzahl der Fische* von Salzgehalt (und Temperatur?) des Meerwassers hingewiesen, die mehrfach bestätigt wurde und sich in Zukunft wohl ebenfalls als "Regel" erweisen wird (vgl. z.B. Schmidt, 1917 1920; Johnsen, 1936). Auch hier handelt es sich zumindest zum Teil um erbliche Merkmale, wie dies u.a. Schnakenbeck (1931) wahrscheinlich machen konnte.

Die bisher in diesem Kapitel genannten Beispiele mögen genügen, um zu verdeutlichen, dass eine durch Aussenfaktoren, vor allem durch klimatische Selektion, gerichtete und damit innerhalb bestimmter Tiergruppen parallele Bildung von Rassen- und Artmerkmalen einen besonders weit verbreiteten Typus darstellt. Es lag nahe, in solchen Fällen einen direkten Einfluss der Umweltfaktoren auf die Ausprägung erblicher Rassen anzunehmen, wie ich dies auch selbst früher (1929 b) voraussetzte. Inzwischen haben nun aber die zahllosen genetischen Untersuchungen erkennen lassen, dass wohl praktisch jedes Gen eine pleiotrope Wirkung hat, dass die Selektion also nicht nur an den klimaparallelen, sondern auch an anderen, damit gekoppelten Merkmalen angreifen kann, wie wir dies bei der Allenschen und Bergmannschen Regel auch z.T. bereits festlegen konnten. So ist heute die Möglichkeit gegeben, richtungslose Mutation und Selektion in jedem Falle als ausreichende kausale Grundlagen auch für diesen Evolutionstyp zu betrachten.—Andere Fälle gerichteter Auslese, die stärkere Formwandlungen bedingen, werden im VI. Kapitel noch besprochen werden.

V. GANZHEITLICHE FORMWANDLUNGEN BEI
RASSEN- UND ARTBILDUNG

Die bisher besprochenen Typen von Rassen- und Artbildung genügen nun aber noch nicht, um das ganze Evolutionsgeschehen einzugliedern. In nicht wenigen Fällen werden zahlreiche morphologische und physiologische Merkmale gleichzeitig von der Umbildung ergriffen, es findet eine "ganzheitliche" Formwandlung statt, die nicht unmittelbar durch Voraussetzung von Mutations- und Selektionsvorgängen zu analysieren ist. So wurde immer wieder die Annahme besonderer immanenter phylogenetischer Entwicklungstendenzen oder gar einer somatogenen Induktion nahegelegt. Derartige Hypothesen finden aber bisher von Seiten der Genetik keinerlei Stütze. Wir müssen uns deshalb fragen, ob sie nicht entbehrlich sind und ob die derzeitigen Erklärungsschwierigkeiten nicht *nur durch die komplexe Verknüpfung der Evolutionsvorgänge* zustandekommen. Ich glaube, dass dies tatsächlich der Fall ist und dass der oft überraschend ganzheitliche Charakter der Rassen- und Artbildung nur dadurch bedingt ist, dass die Selektion bei solchen Formen an der "Ganzheit" angreift und für die einzelnen Evolutionsschritte eine stete Harmonie erzwingt. Von den zahlreichen in Frage kommenden Vorgängen seien hier aber nur die wichtigsten an einzelnen Beispielen behandelt: Pleiotropie, Orthogenese, Kompensation und Umkonstruktion.

(1) *Pleiotropie*

Ein Vergleich der Anzahl bisher—etwa bei *Drosophila melanogaster*—analysierter Gene mit der fast unbegrenzten Zahl der morphologischen, histologischen und physiologischen "Merkmale" macht es verständlich, dass in praxi wohl jedes Gen eine pleiotrope Wirkung hat, auch wenn dies nur erst an einer bestimmten Zahl von Genen erwiesen werden konnte. Es können also bereits durch *einen* Mutationsvorgang sehr komplexe Veränderungen hervorgerufen werden, die den Eindruck einer ganzheitlichen Formwandlung machen. So verursacht z.B. das Gen "Polyphaen" bei *Drosophila funebris* gleichzeitig eine unregelmässige Verteilung und Beborstung der Augenfacetten, eine abnorme Pigmentierung der Abdominalsegmente, häufig auch (Penetranz verschieden) eine Verdickung der hinteren Tergitenränder, gespreizte Flügelhaltung, eine mehrfache Unterbrechung der einzelnen Flügeladern, sowie eine abnorme Ausprägung (meist Reduktion) der Borsten auf Kopf und Thorax (vgl. H. Timoféeff-Ressovski, 1931). Bei der Mehlmotte *Ephestia kühniella* analysierten Strohl und Köhler (1935) ein Gen (dia), das gleichzeitig eine Aufhellung der Pigmentierung, eine Verkürzung der Lebensdauer des Imago und eine Herabsetzung der Fortpflanzungsfähigkeit bedingt. Ähnliche Beispiele sind heute schon in grösserer Zahl bekannt.

Andererseits ist es aber nun leider nicht zu erwarten, dass gerade *die* Formen, die von Seiten der Systematiker als besonders wichtige und markante Typen der Rassen- und Artbildung genannt werden, je in einer ähnlichen Weise analysiert werden. Bei Spechten, Meisen, Raubvögeln, vielen Säugetieren, Eidechsen, *Carabus*-Formen usw. ist wegen der Schwierigkeit der Zuchtbedingungen und wegen

der langsamen Folge der Generationen schon die Vorbedingung, die Herstellung reiner Linien in Bezug auf einzelne Merkmale, praktisch nicht zu verwirklichen. So werden wir hier stets auf Analogieschlüsse angewiesen sein, was umso unbefriedigender ist, als die bestanalyisierten Formen wie *Drosophila* und *Ephestia* in freier Natur kein ausgeprägtes geographisches und ökologisches Variieren erkennen lassen.—Erinnert sei ferner an die pleiotropen Wirkungen von Hormonen, bei denen die geringste mutative Änderung die verschiedensten Merkmale beeinflusst.

Nun dürfen wir zu den pleiotropen Wirkungen aber auch die Verschiebungen hinzurechnen, welche die Ganzheit eines Organismus dadurch erleidet, dass *ein Merkmal die vorher bestehende Harmonie ändert*. Hier können auch wieder morphologische und ontogenetische Untersuchungen stärker zur Klärung beitragen. Wie schon im vorigen Kapitel bei der Besprechung der Bergmannschen Regel auseinandergesetzt wurde, bewirkt z.B. eine erbliche Beschleunigung und Erhöhung der Zellteilungsrate nicht nur eine Vergrößerung des Körpers im ganzen, sondern auch eine Steigerung des Sauerstoffverbrauchs (mehr rote Blutkörperchen bei gleicher Grösse = relativ mehr resorbierende Oberfläche) und eine Veränderung der Proportionen exponierter Körperteile. Da Schwanz, Ohren, Schnäbel und Füsse ein gegenüber dem Gesamtkörper negativ heterogones (über lange Stadien der Ontogenese konstantes) Wachstum haben, führt die Sistierung des Wachstums nach Erreichung der endgültigen Körpergrösse zu einer relativen Verkürzung dieser Organe. Wir haben es also auch hier mit einer ganzheitlichen Änderung zu tun, für deren Deutung wir auf die Annahme unbekannter "innerer" Entwicklungstendenzen verzichten können.

(2) Orthogenese

Noch deutlicher wird dies bei den orthogenetischen Entwicklungsreihen, die ja noch immer als Musterbeispiel für unbekannte phylogenetische Gesetzmässigkeiten angeführt werden. Wie besonders Huxley (1932) durch seine bereits erwähnten, wichtigen Untersuchungen gezeigt hat, ist auch hier in vielen Fällen lediglich die *Berücksichtigung der relativen Wachstumsgeschwindigkeit der einzelnen Organe* unterlassen worden. Als Musterbeispiel können die Titanotherien gelten, deren 4 von Osborn unterschiedene Ahnenreihen sämtlich mit kleinen hornlosen Formen beginnen und mit grossen Formen enden (Abb. 8), die durch riesige frontonasale Hörner ausgezeichnet sind. Hier handelt es sich um ein im Verhältnis zum Gesamtkörperwachstum positiv heterogones, d.h. beschleunigtes Wachstum der Hörner. Mit zunehmender Körpergrösse wurden deshalb die Hörner bei unveränderten Wachstumsgradienten nicht nur absolut, sondern auch noch relativ grösser. In entsprechender Weise dürfte auch die Mehrzahl der übrigen bekannten Excessivbildungen zu deuten sein, so die relativ gewaltige Zahnlänge des Mammut, das "zu grosse" Geweih des Riesenhirsches, die unverhältnismässig grossen Kopf- und Thoraxfortsätze bei grossen Dynastiden und Lucaniden im Verhältnis zu kleineren Arten oder Rassen der gleichen Gattungen usw. (Abbildung bei Huxley, 1932 für 3 Arten von *Golofa*, p. 213, Fig. 94). Es ist also meist nicht

notwendig, solche luxurierenden Organe durch "Abreagieren von Überschüssen in der Ernährungsbilanz" zu deuten, wie dies Krieg jüngst tat (1937), sondern es ist wohl umgekehrt die Ernährungsbilanz von der notwendigen Nahrungszufuhr für die im Vergleich zum Körper beschleunigt wachsenden und in ihrem Wachstumstempo weitgehend konstanten Excessivbildungen abhängig.

Natürlich braucht es sich bei solchen orthogenetischen Reihen nicht immer um eine Steigerung der relativen Grösse bestimmter Organe zu handeln. Ist das Wachstum eines Organs im Verhältnis zum Gesamtwachstum *negativ* heterogon so kann auch eine sukzessive Verkleinerung stattfinden, wie es z.B. bei dem relativen Herzgewicht innerhalb vieler Verwandtschaftsreihen bei Warmblütern der Fall ist.

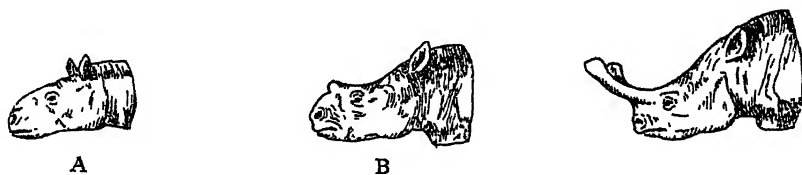


Abb. 8. Drei Formen aus einer Stammesreihe der Titanotherien (nach Osborn) zur Veranschaulichung der Orthogenese infolge positiv heterogonen Wachstums der Hörner. A: *Manteoceras manteoceras* (mittleres Eocän); B: *Protitanotherium emarginatum* (oberes Eocän); C: *Bronthotherium platyceras* (unteres Oligocän).

Da wohl die Mehrzahl der Stammesreihen eine sukzessive Grössensteigerung erkennen lässt (Cope-Déperétsche Regel), so dürfte also auch ein recht beträchtlicher Teil der orthogenetischen Änderungen einzelner Organe in der besprochenen Weise zu deuten sein. Wenn es daneben auch Fälle gibt, die nicht einer Grössenveränderung parallel laufen, so ist zu bedenken, dass weiterhin auch durch zunehmende Klimawandlungen nicht selten die Auslese in bestimmter Richtung eine Steigerung erfährt, dass also eine *Orthoselektion* stattfindet (z.B. im Laufe der sukzessive kälter werdenden Tertiärperiode). Und schliesslich mögen in einigen Fällen schon rein physiologisch die Abänderungsmöglichkeiten so beschränkt sein, dass sie nur in wenigen oder nur in einer Richtung verlaufen können.

Was nun die "orthogenetische" *Grössensteigerung in den Stammesreihen* selbst anlangt, so ist ihre Deutung ebenfalls ohne Zuhilfenahme unbekannter innerer Entwicklungstendenzen möglich. So wies Castle (1932) mit Recht darauf hin, dass grössere erbliche Varianten fast stets die prae- und postnatalen Selektionsvorgänge besser überstehen können als kleinere Varianten, dass damit bereits eine stetige Zunahme der erblichen Grösse gegeben ist. Bei den zahlreichen Säugetier- und Vogelstammesreihen, die sich während des Tertiär entwickelten, kommt dann zum Teil wohl auch noch eine der Bergmannschen Regel entsprechende Grössenauslese durch das sukzessive kühler werdende Klima hinzu (Rensch, 1924).

Schliesslich können manche sukzessiven Formänderungen komplizierterer Natur, die geradezu Musterbeispiele für ganzheitliche Wandlungen sind, ebenfalls überraschend einfach durch Verschiebungen der Wachstumspotenzen im Sinne cartesianischer Transformationen gedeutet werden, wie dies D'Arcy Thompson an Pferdeschädeln, an *Diodon* usw. zeigen konnte (vgl. Huxley, 1932).

Zusammenfassend können wir jedenfalls feststellen, dass heute die Mehrzahl der orthogenetischen Reihen ohne Voraussetzung unbekannter phylogenetischer Tendenzen analysierbar ist.

(3) *Kompensation*

Alle Evolutionsvorgänge, und vor allem auch orthogenetische Änderungen, können nun weiterhin dadurch kompliziert werden und einen noch stärker ganzheitlichen Charakter erhalten, dass sie mit Materialkompensationen verbunden sind. Schon Eimer (1901, p. 7ff., angedeutet bereits 1888) hatte—auf entsprechenden Hinweisen von Geoffroy St. Hilaire und Goethe aufbauend—ein “Gesetz der Ausgleichung oder Kompensation” formuliert, das speziell am Skelett der Wirbeltiere die Ursachen für die Umgestaltung mancher Formen verdeutlichen sollte. Eimer wies vor allem darauf hin, dass mit der Verlängerung der Gliedmassen gewöhnlich eine Verkürzung der Wirbelsäule einhergeht, z.B. bei Fröschen, während umgekehrt eine Vermehrung der Wirbelzahl ein Verkümmern der Gliedmassen zur Folge hat (z.B. bei Schlangen und Schleichen). Indess wurden all diese Fälle nur kurz skizziert, ohne die behaupteten Wechselbeziehungen durch nähere Untersuchungen zu erweisen. Weiterhin hatten auch W. Roux in seiner Hypothese der Intral- bzw. Histonalselektion sowie A. Weismann bei seiner Hypothese der Germinalselektion Kompensationsvorgänge angenommen (Kampf der Determinanten um die Nahrung). Später wurde dann gelegentlich noch bei der Diskussion einzelner Formwandlungen auf entsprechende Erscheinungen hingewiesen, es wurden aber keine Untersuchungen durchgeführt, welche die kompensatorischen Beziehungen ausreichend erhärteten. So verschwand die Kompensation immer mehr aus den stammesgeschichtlichen Diskussionen und es ist interessant, dass z.B. Sewertzoff (1931) bei seiner ausführlichen Darstellung der Reduktion und Rudimentation das Wort überhaupt nicht gebraucht und nur neutral von Koordinationen spricht. Nur Krieg (1937) möchte neuerdings Luxusbildungen als “Abreagieren von Überschüssen in der Ernährungsbilanz” ansprechen. Wie wir sahen, genügt hier zur Erklärung aber in vielen Fällen (hyperteile Bildungen) schon die Beachtung des heterogenen Wachstums. Bei einigen Beispielen (motorisches Luxurieren z.B.) wird dagegen die angedeutete Kompensationserklärung zutreffend sein.

Nun hat Teissier (1934) an den verschiedensten Tieren die wichtige Feststellung machen können, dass die Intensität des Wachstums in jedem Augenblicke proportional der Masse von Geweben ist, die für ein Wachstum zugänglich sind, und zugleich proportional der Gesamtmenge verfügbarer Nährstoffe. Aus dieser Feststellung können wir folgern, dass *Materialkompensation prinzipiell an allen Teilen des Organismus möglich ist.*

Wegen der weitgehenden Bedeutung derartiger Vorgänge für den ganzheitlichen Charakter phylogenetischer Änderungen erscheint es aber notwendig, noch einmal einige neue Beispiele anzuführen, bei denen es sich zunächst um Änderungen innerhalb der gleichen Art, bzw. um entsprechende Differenzen von geographischen Rassen handelt.

Kompensation bei bestachelten und gerippten Schneckenschalen. Bei der Bearbeitung von Süßwasser-Mollusken der Deutschen Limnologischen Sunda-Expedition hatte ich darauf hingewiesen (1934b), dass die bestachelte *Melania scabra* ganz bestimmte Korrelationen der Schalenmerkmale erkennen lässt. Die Spiralstreifen unterhalb des Stachelkranzes sind entweder sehr zart und zahlreich oder mehr oder minder derb und dann viel geringer an Zahl. Durch Prüfung vieler intermediärer Stücke wurde es nun deutlich, dass die Grundzahl der Reifen auf der Unterseite des letzten Umganges stets 22–29 beträgt und dass durch alternierende Verstärkung einzelner Reifen die Skulpturierung der dazwischenliegenden Partien abgeschwächt oder auch völlig aufgehoben wird. Sind sehr kräftige Spiralreifen vorhanden, so beträgt ihre Zahl schliesslich nur noch 7–14. "Es liegt also eine wohl histomechanisch zu deutende Kompensation vor" (Rensch, 1934b, p. 236).

Noch sinnfälliger ist bei *Melania scabra* die *Korrelation zwischen Zahl und Länge der Stacheln* bzw. der Längsrippen, denen die Stacheln aufsitzen. Von 156 untersuchten Schalen zeigten die 58 langstacheligen Exemplare auf dem letzten Umgange 4–10 Stacheln, 54 kurzstachelige Exemplare 7–16 Stacheln und 44 stachellose, schwach längsrippige Exemplare 10–24 Rippen.

Dass auch dieser Korrelation Kompensationsvorgänge zugrundeliegen, veranschaulicht vor allem eine Schale des Berliner Museums von der Insel Saleyer. Hier ist der letzte Umgang zunächst stachellos, lediglich längsrippig. Die letzten Rippen haben einen Abstand von je 1.4 mm voneinander. Dann aber folgen vor der Mündung noch zwei Rippen, die in derbe Stacheln von 2–3 mm Länge auslaufen. Ihr Abstand untereinander und von der letzten stachellosen Rippe beträgt nun aber je 4 mm. Ähnlich verhält es sich mit einer von mir auf Bali gesammelten Schale. Bei dieser sind die Stacheln des letzten Umganges nur etwa $\frac{1}{3}$ mm lang, die drei letzten Stacheln vor der Mündung messen jedoch etwa $1\frac{1}{3}$ mm. Entsprechend sind nun die Abstände der letzten kurzen Stacheln 1.5, 2.2, 2.2 mm, die der längeren Stacheln aber 3 und 3.5 mm. Riech (1937) konnte die Beziehungen zwischen Länge und Zahl der Stacheln auch für die grosse *Melania amarula* bestätigen. Vier in dieser Hinsicht deutlich unterschiedenen Schalen zeigen folgende Durchschnittswerte auf dem letzten Umgange:

Zahl der Dornen	13	10	9	7
Länge der Dornen	2.0	3.0	3.5	4.0 mm

Weitere Beispiele dafür, dass der Mehrverbrauch an Schalenmaterial bei der Ausbildung langer Stacheln ausgeglichen wird, finden sich nun auch bei marinen Mollusken. Auf Abb. 9 sind von 4 Prosobranchier-Arten je 2 extreme individuelle Varianten dargestellt, von denen je die linke lange und wenige, die rechte relativ kurze, aber zahlreicher Stacheln aufweist. So zeigt *Murex (Phyllonotus) radix* Gm. (von Mittelamerika) am letzten Umgang einmal 8 lange, das andere Mal 22 kurze Fortsätze, *Turbo cornutus* Gm. (von Japan) 8 lange oder 14 kurze (4 davon abgebrochen), *Semifusus tuba* Gm. 7 lange oder 10 kurze und *Angaria laciniata* Lam. (aus dem Pazifik) 4 lange (u. 1 kurze) oder 8 kurze Stacheln. So eindeutig, wie bei diesen extremen Varianten sind die kompensatorischen Beziehungen zwischen

Stachellänge und Stachelzahl natürlich nicht in jedem Falle, aber innerhalb der genannten Arten darf die Korrelation jedenfalls doch als bestehend angesehen werden. (Zum statistisch exakten Beweis fehlt es an einer genügenden Anzahl gleich grosser Schalen. Es sei auch betont, dass bei manchen anderen Formen, wie

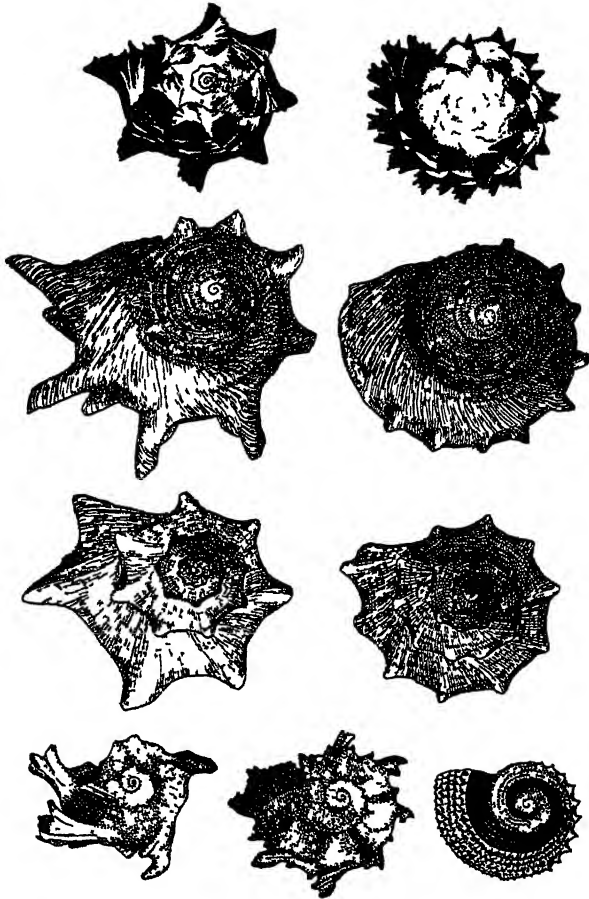


Abb. 9. Meeresschnecken zur Veranschaulichung von Materialkompensation zwischen Stachelzahl und Stachelgrösse. 1. Reihe: gleichgrosse Varianten von *Murex (Phyllonotus) radix*. 2. Reihe: Varianten von *Turbo cornutus*. 3. Reihe: Varianten von *Semifusus tuba*. 4. Reihe: zwei Varianten von *Angaria laciniata* und eine Art mit noch stärkerer Vermehrung der Zahl und entsprechender Verminderung der Grösse der Stacheln: *A. tyria*.

vor allem bei einer Reihe von Muriciden, Stachelzahl und Stachellänge diese wechselseitige Abhängigkeit nicht zeigen.)

Sind nun Grösse und Zahl der Schalenfortsätze erblich bedingt? Und welches der beiden Merkmale ist das Primäre, das die kompensatorische Änderung des anderen nach sich zieht? Genetische Untersuchungen wurden an den genannten Formen bisher noch nicht angestellt. Wir können indess indirekt die Erbllichkeit erschliessen. Die oben erwähnte *Melania scabra* ist nämlich vivipar, und aus dem Uterus herauspräparierte Embryonalschalen zeigen bereits Unterschiede in der

Skulptur. Ist das Muttertier stachelig, so haben die Embryonen auf dem 5. Umgange stumpfe Höcker, ist das Muttertier stachellos, d.h. nur rippig, so zeigt der 5. Umgang nur schwache wellige Erhebungen. Wir können daraus wohl auf Erbllichkeit dieser Merkmale schliessen, denn es ist schwer vorstellbar, dass Umweltseinflüsse, welche die Bestachelung im Wasser aufwachsender Tiere beeinflussen, die gleiche Wirkung auch schon an den Embryonen im Uterus (die physiologisch doch in einer ganz anderen Umwelt leben) haben sollten. Dazu kommt noch, dass bestachelte wie stachellose Formen in den verschiedensten Gewässern zu finden sind, also nicht als Reaktionsformen angesprochen werden können.—Dass gelegentlich aber äussere Umstände den Stachelrhythmus storen können, lehren ja schon die erwähnten beiden Schalen von Saleyer und Bali, bei denen eine Änderung innerhalb einer Schale auftritt. Hier haben wir zugleich auch einen Hinweis darauf, dass der Rhythmus das primäre Merkmal ist, das die Änderung der Stachelnlänge nach sich zieht, denn es wurde an diesen Schalen beim allmählichen Wachstum zeitlich zuerst der Abstand vergrössert (Saleyerschale: 4 mm, statt 1·4 mm) und dann erst der Stachel verlängert (von 0 auf 2 mm).

Ähnliche Kompensationserscheinungen finden sich auch bei manchen *Land-schnecken*. Es gibt hier eine ganze Anzahl von Formen, bei denen glattschalige und gerippte individuelle Varianten oder geographische Rassen auftreten, oder es liegen zwei nächstverwandte Arten vor, die sich im Schalenbau hauptsächlich durch Vorhandensein oder Fehlen der Rippung unterscheiden. In solchen Fällen pflegt dann zumeist die *gerippte Form*, die also Schalenmaterial für ihre Skulptur verwendet, *etwas kleiner* zu sein. Da mir nicht ausreichendes Material vorliegt, um die statistische Realitäts solcher Unterschiede zu erweisen (starke individuelle und ökologische Grössen-Variabilität), so möge es genügen, hier nur kurz zwei Beispiele anzuführen. Die glattschalige, sudalpine Felsenschnecke *Campylaea c. colubrina* Jan., von der ich 105 Exemplare des Berliner Zoologischen Museums messen konnte, hat eine Höhe von 10·0–16·2, durchschn. 12·8 mm und einen Durchmesser von 18·6–28·3, durchschn. 23·5 mm. Im Val Vestino wird diese Art vertreten durch die derb radiär gerippte *C. colubrina gobanzi* Frfd., die (auf Grund 77 gemessener Exemplare) eine Höhe von 10·0–15·6, durchschn. 12·4 mm, und einen Durchmesser von 19·6–27·7, durchschn. nur 22·8 mm hat.—Die grosse *Helix a. aspersa* bildet in Westsizilien eine ökologische Rasse *maxzullii* aus, die gerippt und "infolgedessen" etwas kleiner ist. Sie hat eine Höhe von 25·4–36·6, durchschn. 30·5 mm und einen Durchmesser von 21·7–33·2, durchschn. 28·1 mm (97 Exemplare des Berliner Museums gemessen), während die Normalform auf Sizilien 26·8–35·6, durchschn. 31·7 mm hoch ist und einen Durchmesser von 26·6–36·7, durchschn. 32·6 mm aufweist (18 Schalen gemessen).

Dass es sich in diesen beiden Fällen bei der Ausbildung der Rippen nicht um eine Mehrproduktion von Schalensubstanz handelt, lehren auch die Gewichtszahlen. Für *Campylaea col. colubrina* konnte ich ein durchschnittliches Schalengewicht von 0·49 g feststellen¹ und für die rippige *C. col. gobanzi* von 0·45 g. *Helix a.*

¹ Es wurden nur solche Schalen zur Wägung benutzt, die sich bei durchscheinendem Licht (Lampe von 100 Watt) bis in die Embryonalwindungen hinein als frei von Fremdkörpern erwiesen. Das war der Fall bei 60 *colubrina*- und 55 *gobanzi*-Schalen.

aspersa-Schalen wogen durchschnittlich 1.97 g (15 Exemplare gewogen), *H. a. mazsullii*-Schalen 1.40 g (74 Exemplare gewogen). Es sind also die gerippten Formen in beiden Fällen sogar leichter als die ungerippten, d.h. die Schalenpartien zwischen den Rippen müssen ganz besonders dünn sein. Tatsächlich ist dies auf quer zu den Rippen verlaufenden Schliffen deutlich zu erkennen. *Die Rippen entstehen also auf Grund einer Materialkompensation.* Auch bei den Landschnecken sind Rippung und Glattschaligkeit wohl als erblich bedingt anzusprechen. Dafür spricht die Tatsache, dass sich bei Kreuzung einer glatten und einer gerippten Rasse der sizilischen Landschnecke *Murella muralis* diese beiden Charaktere als erblich erwiesen.¹

Kompensation bei Schwungfedern von Vögeln. Im IV. Kapitel wurde eine "Flügelschnittregel" besprochen, die besagt, dass innerhalb eines Vogelrassen-

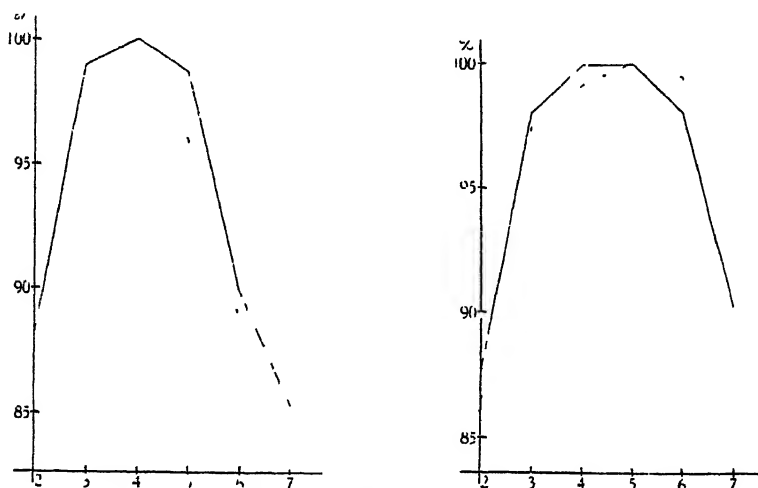


Abb. 10. Flügelschnittkurven zur Verdeutlichung der Materialkompensation von Handschwingen vor und hinter der Flügelspitze. Links: *Lanius exc. excubitor* (ausgezogen) und *L. exc. elegans* (punktiert). Rechts: *Regulus reg. regulus* (ausgezogen) und *R. reg. teneiffae* (punktiert). Ordinate: durchschnittliche Entfernung der Federspitze vom Flügelbug in % der Flügellänge. Abscisse: Zahl der Handschwingen. (Zahlen bei Rensch, 1934c.)

kreises die Rassen kühlerer Gebiete im allgemeinen eine spitzere Flügelform haben als Rassen wärmerer Klimate. Diese Zuspitzung des Flügels kommt dadurch zustande, dass die Schwungfedern, welche die Flügelspitze bilden, verlängert sind auf Kosten der benachbarten Handschwingen. Für die genauen Messungen sei auf die Zahlen und Diagramme meines Vortrages in Oxford (Rensch, 1934c) verwiesen. Hier möge es genügen, zwei in der genannten Arbeit nicht dargestellte Diagramme zu bringen (Abb. 10). Berücksichtigt sind dabei die 2.-7. Handschwinge, d.h. die 6 Federn, welche zumeist die Spitze des Flügels bilden. Es wurden jeweils die Abstände vom Flügelbug bis zur Federspitze gemessen und von mehreren Exemplaren gleichen Geschlechtes Durchschnittswerte für jede einzelne Feder errechnet. Da nun die absoluten Masse nicht vergleichbar sind, weil die Vogelrassen verschie-

¹ Für Messungen sind diese Formen ungeeignet, da die gerippte Rasse eine ganz andere Form hat (sie ist flach und gekielt; vgl. Rensch, 1937).

den gross sind, wurde berechnet, wieviel Prozent der gesamten Flügellänge jede Federspitze im Durchschnitt erreicht. Diese Werte wurden auf der Ordinate aufgetragen, während die Abscisse die Zahl der Schwungfedern angibt. Die Abb. 10 zeigt nun, dass bei *Lanius e. excubitor* von Livland gegenüber *L. e. elegans* Sw. von NW. Africa die 2. Schwinge stark, die 3. schwächer relativ verkürzt ist, dass "dafür" aber die 4.-7. Schwinge relativ etwas verlängert sind. Beim Goldhähnchen liegen die Verhältnisse dagegen umgekehrt: hier sind bei der nördlicheren Rasse *Regulus r. regulus* L. (von Polen und Livland) die 2., 3. und 4. Schwinge relativ verlängert, dafür sind aber die 6. und 7. Schwinge gegenüber der südlichen Rasse *teneriffae* stärker verkürzt. Bei diesen zu gleicher Zeit wachsenden Handschwingen findet also eine Kompensation in der Weise statt, dass eine Verlängerung der Federn vor der Flügelspitze eine relative Verkürzung hinter der Spitze bedingt und umgekehrt. Ganz Entsprechendes zeigen auch die hier nicht abgebildeten Diagramme von *Parus a. ater*-*P. a. ledoucii*, von *Parus c. caeruleus*-*P. c. palmensis*, von *Parus m. major*-*P. m. cinereus*, von *Parus palustris crassirostris*-*P. p. italicus*, von *Phylloscopus collybita tristis*-*Ph. c. canariensis*, von *Acrocephalus a. arundinaceus*-*A. a. meyeri*, von *Cisticola j. juncidis*-*C. j. fuscicapilla*, von *Fringilla c. coelebs*-*F. c. koenigi*, von *Passer d. domesticus*-*P. d. indicus* u.a. Doch sind die Rassen natürlich nur dann gut vergleichbar, wenn die Masse der beiden Rassen nicht zu stark unterschieden sind (wie das z.B. bei den zuerst besprochenen *Lanius*- und *Regulus*-Rassen der Fall ist).

Da die Zuspitzung des Flügels im kühleren Gebiete meist durch eine relative Verlängerung der 2.-3. (sehr selten wie bei *Lanius excubitor* der 5. und 6.) Schwinge bedingt ist, ist auch fast regelmässig die meist ohnehin schon rudimentäre 1. Schwinge stärker verkürzt. Besonders auffällig ist dieser Unterschied auch bei Formen, die schon als Arten gelten müssen: bei dem östlichen Sprosser (*Luscinia luscinia*) mit spitzerem Flügel und sehr kurzer 1. Schwinge und der mehr westlich und südlich verbreiteten Nachtigall (*Luscinia megarhynchos*) mit rundlicherem Flügel und längerer 1. Schwinge. Da beide Formen im Grenzgebiet unvermischt nebeneinander leben (zwischen Oder und Weichsel), können wir hier auch die Erblichkeit des Merkmals als erwiesen betrachten. Zugleich sehen wir an diesem Beispiel, dass die ganzheitlichen kompensatorischen Beziehungen der Schwungfedern in gleicher Weise für Rasse- wie für Artbildung Bedeutung haben.

Zumeist werden solche Materialkompensationen bei topographisch benachbarten Organen auftreten und man könnte hier noch eine ganze Anzahl weiterer Beispiele nennen (z.B. Reduktion der Polzellen nur bei weiblichen, nicht bei männlichen Keimzellen; Reduktion von schwächeren Keimzellen in den Gonaden; man vgl. auch die unten genannten Fälle von Rudimentation). Aber die ganzheitlichen Verknüpfungen innerhalb eines Tierkörpers sind so weitgehend, dass auch topographisch ferne Organe einander derart beeinflussen können. Ich möchte hier als Beispiel die von Ramme (1931) aufgedeckte Tatsache erwähnen, dass bei manchen Orthopteren ein unmittelbarer Zusammenhang zwischen Flügellänge und Keimdrüsenausprägung besteht. Es gibt nämlich bei normalerweise brachypteren Arten (wie *Metrioptera roeselii*) stellenweise auch langflügelige Exemplare: bei diesen waren aber, soweit es sich um Weibchen handelte, stets die Ovarialschläuche

vollig reduziert und enthielten nur kleine schlaffe Eier. Es liegt nahe anzunehmen, dass hier die physiologische Belastung des Körpers durch Ausbildung der grossen Schwingen zur Reduktion des Genitalapparates führte. Zu beweisen ist diese Annahme freilich noch nicht. Und auch die vielen Fälle pleiotroper Genwirkung sind wahrscheinlich z.T. auch nur ein Ausdruck derartiger kompensatorischer Beziehungen im Gesamtorganismus, was aber nur beweisbar ist, wenn es gelingt, alle anderen Möglichkeiten gegenseitiger Abhängigkeit (besonders solche auf hormonaler Grundlage) auszuschliessen.

Nun ist die Materialkompensation aber auch noch in anderen Beziehungen für die Frage der Artbildung von Bedeutung. In erster Linie gilt das für das *Problem der Rudimentation*. Wenn in einer Ahnenreihe ein Organ allmählich reduziert und schliesslich funktionslos wird, so machte die Deutung dieses Vorganges nur durch Selektionsvorgänge gewöhnlich Schwierigkeiten (mangelnder Selektionswert geringer Verkleinerungen), und es ist verständlich, wenn gerade bei vergleichend-anatomischen Untersuchungen so oft eine lamarckistische Erklärung versucht wurde. Diese Schwierigkeiten fallen aber fort, wenn die Materialkompensation berücksichtigt wird. Für jedes Organ sind ja Zellgrösse und Teilungsrate erblich fixiert. Erhöht sich nun mutativ die Teilungsrate eines Organs, so kann prinzipiell stets eine Kompensation stattfinden, weil für die übrigen (besonders die topographisch benachbarten) Organe nun die Gesamtmenge verfügbarer Nährstoffe geringer ist, und nach Teissier (1934, s.o.) ist ja die Intensität des Wachstums in jedem Augenblick proportional der Gesamtmenge verfügbarer Nährstoffe. Reduktion und Rudimentation wird also gewöhnlich mit dem Anwachsen anderer Organe parallel laufen. (Natürlich ist das nicht immer so: es ist auch Reduktion durch Wegfall der auslösenden Gene möglich, wie Sewertzoff, 1931 rein ontogenetisch an einigen Beispielen zeigte.)

Vergleichen wir z.B. die einzelnen Stadien bei der Reduktion der Zehen in der bekannten "Pferdereihe": in dem Masse, wie der 3. Zeh kräftiger und absolut und relativ länger wird, werden die übrigen Zehen zurückgebildet, bis schliesslich bei *Equus* nur noch die "Griffelbeine" als Rudimente der 2. und 4. Zehe übrigbleiben. Wenn wir nun diese Rudimentation nicht durch einen (kaum erklärbaren) Fortfall der Anlagen für die 2. und 4. Zehe deuten wollen, sondern durch Materialkompensation innerhalb der gleichzeitig wachsenden, topographisch benachbarten Zehen, so würde dies bedeuten, dass die Gene für die Ausbildung der an den Griffelbeinen fehlenden Phalangen noch vorhanden sind. Das ist nun aber tatsächlich der Fall. Bei Pferden tritt gelegentlich eine Polydactylie auf, bei der der 2. Zeh eine ganz normale Phalangenzahl besitzt. Besonderes Interesse hat hier z.B. ein von Ružička (1933) beschriebener Fall, der deutlich zeigt, dass die 3. Hauptzehe zugleich *schwächer* ausgebildet ist. Die Ausbildung der mehr oder minder normalen 2. Zehe liegt also bei Pferden im Bereiche der Reaktionsnorm, wird aber normalerweise durch Materialkompensation verhindert.

Wenn eine solche Erklärung für einen Teil der Fälle von Rudimentation (nicht generell, s.o.) zutrifft, dann ist zu erwarten, dass *die Rückbildungen besonders dann schnell vor sich gehen, wenn die Wachstumsperiode der Rudimente mit der Hauptwachs-*

tumsperiode benachbarter (oder allgemein in einer Einflussphäre liegender) Organe zusammenfällt. Die Phalangen der Huftiere werden z.B. in der Hauptstreckungsphase der Extremitäten ausgebildet, sie erfahren deshalb eine schärfere Reduktion als die sich zeitiger entwickelnden Metacarpalia und Metatarsalia. Deshalb beginnt die Reduktion ganz allgemein am distalen Ende der Extremitäten. Bei der mit der Verlängerung der Wirbelsäule zunehmenden Reduktion der Extremitäten von Reptilien können wir ganz das Gleiche beobachten (vgl. Sewertzoff, 1931, Kap. XII, und die instruktive Tafel hinter p. 220).

Umgekehrt bleiben aber Rudimente dann besonders lange erhalten, wenn eine unmittelbare anderweitige Verwendung des dafür notwendigen Materials nicht in Frage kommt. So ist es vielleicht zu verstehen, dass die Becken- und Oberschenkelrudimente bei manchen Walen sich so lange erhalten konnten. Hingewiesen sei auch auf den meist rudimentären Eckzahn im Oberkiefer der Wiederkäuer, der wohl erhalten blieb weil keine Schneidezähne als histomechanische Konkurrenten ausgebildet werden, während andererseits bei den Nagern mit ihren grossen Schneidezähnen die Eckzähne völlig verschwanden. Die Kompensationsvorgänge werfen somit auch Licht auf die Frage, *warum manche Strukturen, die für die Ausgangsformen sehr bedeutungsvoll waren, im Laufe der Ontogenese verschwinden, während andere Strukturen, die offenbar stets unwichtig sind, immer wieder rekapituliert werden.* Es wird zu untersuchen sein, ob nicht diese letzteren ("taxonomisch wichtigen") Merkmale gewöhnlich solche sind, die von einer Materialkompensation nicht so leicht erfasst werden (Problem der Abkürzung der Rekapitulation).

Materialkompensationen können nun auch innerhalb eines Organes auftreten und dann besonders für das Zustandekommen eines *Funktionswechsels* Bedeutung haben. Es sei hier nur ein Beispiel genannt, bei dem die Verhältnisse relativ klar liegen und das uns zugleich zeigt, wie eine genetische Anlage vorhanden sein kann, ohne sich normalerweise je zu manifestieren. Die *Radien an den Schillerradien* der meisten Vögel bilden eine Kette flacher, rechteckiger, verhornter Zellen. Die normale Differenzierung in Haken- und Bogenradien, d.h. die Ausprägung von Haken, Dornen und Rinnen fehlt dabei. Nun treten bei Schillervögeln wie z.B. beim Pfau oder bei unserem Star gelegentlich Albinos auf. Die Radien solcher Exemplare (Abb. 11) sind nun aber nicht nur durch den Mangel von Pigment charakterisiert, sondern auch dadurch, dass ganz normale Haken- und Bogenradien ausgebildet sind (Rensch, 1925). Ich nahm damals an, dass diese Koordination der starken Pigmentierung mit einem Mangel der Differenzierung von Haken und Dornen auf ein ursächliches Verhältnis zurückzuführen sei, dass nämlich das Eindringen grosser Melaninmassen die Radiuszellen automatisch verbreitert, die Ausbildung der Haken unmöglich macht und den Schiller durch Ausziehen der obersten Hornschicht zu einem dünnen Blättchen (Interferenz) hervorruft. Diese Hypothese wurde später durch experimentelle Erzeugung von stärkerer Pigmentierung und zugleich von typischen Schillerradien durch Neunzig bestätigt. So haben wir hier also ein Beispiel von Materialkompensation innerhalb eines Organs (Verbrauch der Hakensubstanz für die breiten Schillerzellen), die zugleich einen Funktionswechsel bedingt: die Verankerung der Federradien untereinander wird

unmöglich, die Feder ist flugmechanisch weniger oder nicht wirksam (daher an den Handschwingen sehr selten schillernde Partien).

Schliesslich sei auch noch kurz angedeutet, dass ebenfalls die Entstehung der *Färbungs- und Augenrudimentation bei Höhlentieren* durch Annahme von Kompensationsvorgängen leichter verständlich wird. Kosswig (1937 a, b) hat kürzlich an Wasserasseln sehr schön die Phasen der Reduktion und ihre Erblichkeit nachweisen können. Seine Deutung des Zustandekommens einheitlich unpigmentierter und blinder Höhlentiere durch wiederholtes Auftreten der gleichen Verlustmutation und durch das Prinzip fortgesetzter Elterneinschränkung kann man aber wohl noch nicht als befriedigende Erklärung betrachten. Man wird vielmehr die Frage aufwerfen müssen, warum es denn gerade immer wieder die typischen "Höhlenmerkmale" sind, die im Dunkeln angereichert werden, warum nicht auch andere Verlustmutanten in Frage kommen. Setzen wir dagegen voraus, dass in Höhlen Varianten begünstigt sind, die andere Organe kompensatorisch auf Kosten der Augen oder der Pigmentierung verbessern, so haben wir es mit einem positiven Selektionswert zu tun, der die Einheitlichkeit der Höhlenmerkmale, d.h. die Reduktion der hier unwichtigen Merkmale erklären würde. Kurz gesagt: Erhaltenbleiben von Verlustmutanten und Elterneinschränkung bei kleinen Populationen genügen, um eine erhöhte Variabilität verständlich zu machen, für die Einheitlichkeit von Pigment- und Augenlosigkeit bei Höhlentieren muss aber ein positiver Selektionswert hinzukommen, der mit dem Verbrauch des "gesparten" Materials an anderer Stelle (Tastorgane etc.) gegeben wäre. Die Tatsache, dass der Grottenolm (*Proteus*) die Fähigkeit hat, im Licht wieder Augen auszubilden, spricht wohl auch für eine solche pleiotrope bzw. polygene kompensatorische Bedingtheit der Augen.



Abb. 11. Federradien von den Oberschwanzdecken des Pfaues (*Pavo cristatus*). Links: "normaler" Hakenradius eines albinotischen Vogels; rechts: Schillerradius eines normal pigmentierten Vogels. (Nach Rensch, 1925.)

(4) Umkonstruktionen im Sinne Bökers

Mit Bökers erfolgreicher "Einführung in die vergleichende biologische Anatomie der Wirbeltiere" (1935, 1937), wurde das anatomische Studium auf eine neue Grundlage gestellt, weil hier alle anatomischen Konstruktionen prinzipiell im Rahmen der körperlichen Ganzheit untersucht wurden. Es ergab sich dabei, dass phylogenetische "Anpassungsreihen", die mit einiger Vorsicht auch an "anatomischen Reihen" rezenter Tiere studiert werden können, im allgemeinen nicht durch Abänderungen eines einzelnen Merkmales, sondern durch "Umkonstruktionen" zustandekommen, bei denen viele Merkmale als funktionelle Gesamtheit

den Umweltsverhältnissen angepasst werden. Böker folgert daraus, dass hier ein "sinnvolles", "aktives Reagieren" vorliege, also ein Vorgang, für den eine Deutung durch "passives Zufallsgeschehen durch Mutation und Selektion" nicht genügt (Böker, 1936). Wenn nun ein solches aktives Reagieren eines Organismus phänotypisch z.T. sehr wohl nachweisbar ist, so fehlt uns doch bisher noch jede Grundlage für die Annahme eines entsprechenden phylogenetischen Reagierens, d.h. eines Reagierens der Erbmasse bzw. der dazu notwendigen somatogenen Induktion.

Meines Erachtens sind aber die Folgerungen Bökers (denen ich früher selbst zustimmte) heute nicht mehr nötig. Wie in diesem Kapitel unter (1) bis (3) gezeigt wurde, gibt es eine grosse Zahl ganzheitlicher Änderungen, die auf der Grundlage von Selektion und Mutation zustandekommen können und viele dieser Änderungen können durchaus als "Umkonstruktionen" angesprochen werden. Die mutativ möglichen komplexen Wandlungen infolge Verschiebung der Wachstumsheterogonie, die dabei möglichen cartesianischen Transformationen, die Materialkompensationen und schliesslich die Pleiotropie der meisten Gene ganz allgemein vermögen wahrscheinlich im Zusammenwirken mit natürlicher Auslese in jedem analysierbaren Falle eine Umkonstruktion kausal zu klären. Schon jede mutative Änderung der Gesamtgrösse bedingt ja, wie wir sahen, eine automatische Verschiebung fast aller Organproportionen (Wachstum im Vergleich zum Gesamtkörper heterogon) und gibt dadurch für die Auslese neue Angriffsmöglichkeiten. Es sei hier nur auf ein von Böker (1937, Bd. 2, p. 34) abgebildetes Beispiel eingegangen. Die Schädel der Schweine *Potamochoerus*, *Hylochoerus* und *Phacochoerus* bilden eine anatomische Reihe für "sich steigernde Ausbildung des Wühlens mit den Hauern des Oberkiefers" (Abb. 12). Da die Schädel aber auch eine zunehmende Grösse zeigen, können wir die relative Zunahme der Hauergrösse auch durch die damit verbundene Auswirkung eines positiv heterogenen Wachstums der Hauer erklären (also ganz so wie die Zunahme der Titanotherien-Hörner, s.o.) und die Einschnürung des Schädels hinter den Hauern könnte auf Materialkompensation zurückgeführt werden. Zumindest kann man sagen, dass eine einzige Mutation, welche eine Steigerung der Gesamtgrösse eines Flusschweines bedingt, anatomische Änderungen in einer solchen Richtung mitbedingen würde.

Im übrigen aber ist die Ganzheitlichkeit der Umkonstruktionen zumeist schon damit erklärbar, dass *die natürliche Auslese ja immer die Ganzheit des Individuums trifft*. Bei Änderung der Nahrung von Vögeln z.B. wird nicht eine neue Kropfform ausgelesen, sondern eine neue Variante des Tieres, bei dem nicht nur eine andere Kropfform vorliegt, sondern bei dem diese neue Kropfform in Harmonie zur Ausbalanzierung des gesamten Körpers steht. Mit einer Selektionserklärung sehen wir also Ursache und Folge umgekehrt als Böker. Wenn Böker z.B. schreibt (1936, p. 274): Ursache: harte Pflanzennahrung; Folge: Kropf S-förmig und sehr gross, so würde man wohl richtiger umgekehrt sagen müssen: Ursache: mutativ grösserer Kropf; Folge: Möglichkeit, sich nun auch auf harte Pflanzennahrung umzustellen. Und wenn Böker weiter schreibt: Vordergewicht durch den grösseren Kopf zu gross; Folge: grösserer Tragflügel, längerer Schwanz, Verlagerung des

Kropfes in den Schwerpunkt, so wurden wir wiederum umgekehrt sagen: unter den Varianten mit grosserer Kropfform sind nur solche dauernd erhaltungsfähig, die zugleich grossere Tragflügel, grosseren Schwanz und andere Lage des Kropfes aufweisen. Diese Selektionserklärung setzt lediglich voraus, dass *alle* Organe erbliche Varianten aufweisen, und das trifft generell zu, und weiterhin, dass die Auslese stets die Ganzheit des Individuums trifft, und auch das dürfte nicht zweifelhaft sein. Solange aber die Erklärung mit bekannten Tatsachen der Genetik genügt, werden wir die Annahme eines bisher unerklärbaren aktiven genetischen Reagierens vermeiden müssen.

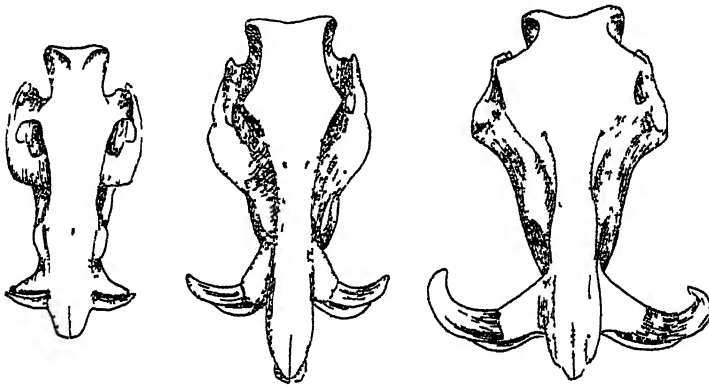


Abb. 12. Umkonstruktion von Schweineschädeln zur Veranschaulichung positiv heterogenen Wachstums der Hauer. Links: *Potamochoerus*; Mitte: *Hylochoerus*; rechts *Phacochoerus* (nach Boker, aus Brehm, *Tierleben*).

(5) Bedeutung für die Systematik

Es ist bei manchen Tierformen schwer zu entscheiden, ob es sich um eine besondere Art oder um eine individuelle Variante handelt. Die Systematiker pflegen in solchen fraglichen Fällen die Aufstellung einer besonderen Art dann für berechtigt anzusehen, wenn die Differenzen mehr als ein Merkmal betreffen. Die in diesem Kapitel besprochenen Fälle lehren uns nun aber, dass in vielen Fällen schon durch einen Mutationsschritt infolge heterogenen Wachstums der einzelnen Organe, infolge von Kompensationserscheinungen bzw. infolge der pleiotropen Genwirkung ganz allgemein viele Merkmale zugleich abändern können. Speziell ist mit jeder Grössenveränderung automatisch eine Verschiebung der Proportionen verbunden, da die einzelnen Organe praktisch niemals sämtlich ein hinsichtlich der Gesamtgrösse isogones Wachstum aufweisen. Die Systematiker müssen deshalb in Zukunft bestrebt sein, diese ganzheitlichen Bindungen zu erkennen (wie das z.B. oben bei der Bergmannschen Regel erläutert wurde) und zumindest bei evolutionistischen oder phylogenetischen Folgerungen nicht nur die Differenzen von Einzelmerkmalen zu berücksichtigen. Schliesslich wird auf diese Weise auch die Schaffung immer wieder neuer Synonyme vermieden (man vergleiche etwa die Synonymik von Arten, die kompensatorische Beziehungen, wie

von Stachelzahl und Stachellänge bei Schnecken aufweisen: entsprechende Varianten der oben erwähnten *Melania scabra* z.B. wurden unter 10 verschiedenen Namen beschrieben!).

VI ENTWICKLUNG HOHERER KATEGORIEEN

In den letzten Jahren ist von Berg (1926), Philpitschenko (1927), Rosa (1931), Goldschmidt (1932, 1935), von Bertalanffy (1937), Beurlen (1937) u.a. aus den verschiedensten Erwägungen heraus immer wieder die Annahme vertreten worden, dass die Evolution von Arten, Gattungen und höheren Kategorieen anderen, z.T. noch völlig unanalysierten Gesetzmäßigkeiten folge als die Bildung von Rassen. Da nun aber bisher niemals mutative Sprünge beobachtet wurden, die unmittelbar zu neuen Arten führten, so ist eine solche "Makroevolution" an sich schon unwahrscheinlich. Nun soll damit allerdings wohl weniger ein Überspringen des Rassenstadiums behauptet werden, als vielmehr eine Sondergesetzmäßigkeit bei denjenigen Ahnenreihen oder Rassenketten, die nachweisbar zu grösseren Transformationen und vor allem auch zu einer Form der "Höherentwicklung" führen. Am wichtigsten sind hier wohl die Sondergesetzmäßigkeiten, welche die paläontologischen Untersuchungen in eindeutiger Weise klarlegten. Wir wollen uns deshalb auf die Besprechung dieser wichtigen Befunde beschränken und uns die Frage vorlegen, ob wir es hier tatsächlich mit besonderen Typen der Rassen- und Artbildung zu tun haben, die sich den bisher besprochenen Fällen nicht einfügen lassen.

Die hauptsächlichsten Sondererscheinungen, welche das Studium grosserer Entwicklungsreihen erkennen liess, sind folgende: die Irreversibilität der Entwicklung, die Orthogenese, die stete Grössenzunahme innerhalb der Entwicklungsreihen, die Erscheinungen der explosiven Formenaufspaltung, des nachfolgenden Alterns und Aussterbens der Entwicklungsreihen und schliesslich die viel diskutierte "Höherentwicklung".

(1) Irreversibilität

Das von Dollo (1893) aufgestellte "Gesetz", dass die phylogenetische Entwicklung nie wieder zu einem früheren Stadium zurückkehrt, hat sich an den verschiedensten Ahnenreihen immer wieder bewahrheitet. Wir müssen uns aber darüber klar sein, dass diese Regel nur für grössere Entwicklungsschritte und nur deshalb zutrifft, weil es sich gewöhnlich um eine Fülle von mutativen Änderungen handelt und weil sich zumeist auch die Umwelt der Ahnenreihe zeitlich oder räumlich allmählich ändert, sodass neue Auslesebedingungen zustandekommen. Es ist also nur die Kompliziertheit der Evolutionsbedingungen, welche es unwahrscheinlich macht, dass die Erbmasse wieder auf ein früheres Stadium zurückgeführt wird. Für kleine Entwicklungsschritte ist mit dem eindeutigen Nachweis von Rückmutationen (vgl. N. W. Timoféeff-Ressovsky, 1937) die Irreversibilität widerlegt. Und bei dem pleiotropen Charakter der meisten Gene ist damit auch die gleichzeitige Rückentwicklung vieler "Merkmale" durchaus möglich. Es besteht also heute kein Anlass mehr, mit Beurlen (1937) die Irreversibilität als "Ausdruck eines gesetzlich sich aufbauenden Gestaltungsplanes" anzusehen.

(2) *Orthogenese*

Im V. Kapitel wurde bereits gezeigt, dass die Beachtung des positiv oder negativ heterogenen Wachstums der einzelnen Organe bei Grössenänderungen und die Berücksichtigung einer oft mit den parallelen Auslesebedingungen gegebenen "Orthoselektion" gerade die auffälligsten Beispiele orthogenetischer Entwicklung zu erklären vermögen. Mutation und Selektion genügen auch hier als letzte Voraussetzungen. Man sollte deshalb auch bei Beispielen, die bisher in dieser Weise noch nicht analysiert wurden, eine Deutung auf der gleichen Grundlage versuchen. Zu berücksichtigen ist dabei vor allem auch die Tatsache, dass viele orthogenetische Reihen nicht als geradlinige Ahnenreihen gegeben sind, sondern dass Seitenzweige der Entwicklung dabei *vernachlässigt* wurden und zum Teil auch noch nicht bekannt sein können.

(3) *Cope-Dépérétsche Regel der Grössenzunahme innerhalb der Stammesreihen*

Auch für diesen Sonderfall der Orthogenese scheint mir die im V. Kapitel besprochene Deutung durch natürliche Auslese grosswüchsiger Varianten und durch Wirksamkeit der Bergmannschen Regel in der Zeitfolge (z.B. kühler werdendes Tertiär) ausreichend zu sein, um von der Annahme eines immanenten Entfaltungsprinzips absehen zu können. Zu berücksichtigen ist dabei auch die schon bei der Bergmannschen Regel besprochene Tatsache, dass grössere Formen wegen der zunächst gegebenen Konstanz der Zellgrösse nicht grössere, sondern *mehr* rote Blutkörperchen haben, dass also die resorbierende Oberfläche nicht proportional geblieben, sondern relativ erhöht ist und dass somit auch diese Rationalisierung des Stoffwechsels einen Vorteil bietet, der die Auslesebedingungen begünstigt (leichteres Überdauern, wenn die Stoffwechselbedingungen durch Umwelteinflüsse ins Minimum geraten).

(4) *Explosive Formaufspaltung, Überspezialisierung und Aussterben von Ahnenreihen*

Es gehört zu den wichtigsten Entdeckungen der Paläontologie, dass die Entstehung neuer Gruppen meist durch eine explosive Formenaufspaltung eingeleitet wird, die dann sukzessive abklingt, während zugleich die Spezialisierung der einzelnen Formen immer mehr zunimmt, bis schliesslich alle oder einige der Stammesreihen mit überspezialisierten bzw. excessiven Formen ausstirbt. Es ist verständlich, wenn ein derart regelmässiger Ablauf der Phylogenese als Ausdruck eines immanenten Entfaltungsplanes angesehen wird (vgl. z.B. Beurlen, 1937). Wir müssen indes doch versuchen, auch diesen "Entfaltungsplan" möglichst mit Hilfe bekannter Tatsachen, speziell mit Mutation und Selektion zu klären.

Wir wissen heute, dass die Mutation zunächst richtungslos ist, dass ihr praktisch alle Allele, wenn auch in verschiedenem Grade unterworfen sind. Die Mutationsrate ist weiterhin abhängig von der Temperatur und unter Umständen von der Einwirkung harter Strahlungen. Es wäre also denkbar, dass eine explosive Formauf-

spaltung mit geologischen Perioden zusammenfällt, in denen harte Strahlungen (in Frage kommen wohl hauptsächlich nur ultraviolette Strahlen) in stärkerem Masse die Erdoberfläche erreichten oder in denen das Klima wärmer war. Die stärkere Formenneubildung in den Tropen (hier allerdings zugleich mehr Generationen im Jahr) und vielleicht auch bei Warmblütern könnten als Beispiele genannt werden. Generell trifft aber eine solche Erklärung nicht zu: explosive Formenaufspaltung findet sich vielmehr praktisch in fast jeder geologischen Epoche.

Bei konstanter Temperatur ist nun die Mutation als zeitproportional erkannt, d.h. sie ist ein stetiger Prozess (vgl. N. W. Timoféeff-Ressovsky, 1937). Da jede Umwelt jederzeit auch selektionierend wirkt, *so bedeutet eine Stetigkeit der Mutation zunächst, dass einer Isolation von Populationen eine evolutionsfördernde, nicht nur (wie bisher angenommen) eine erhaltende Wirkung zukommt.* Vor allem wird es damit wahrscheinlich, *dass explosiver Beginn und späteres Abklingen der Formenneubildung wahrscheinlich weniger auf Änderung der Mutationsrate als auf Änderung der Selektionsbedingungen zurückzuführen ist.*

Führt die Evolution zu neuen vorteilhaften Typen wie etwa zur Entstehung von Säugetieren, so stehen für diese zunächst praktisch alle Biotope zur Verfügung, denen sie selektiv angepasst werden können, d.h. es kann eine schnelle und vielseitige Artbildung einsetzen. So ist es zu verstehen, wenn schon die Beuteltiere "schnell" aufspalten, laufende, kletternde, flatternde, springende und wühlende, räuberische, insektenfressende, pflanzenfressende und nagende Typen hervorbringen. In dem Masse, wie die Biotope aber mit der neuen Tiergruppe erfüllt werden, wird die Konkurrenz immer schärfer und trotz stetiger Mutation muss die Formenneubildung wegen verschärfter Selektion nachlassen. Als sich dann die "rationeller konstruierten" Plazentaltiere bildeten, wiederholte sich die gleiche Situation: die Beuteltiere konnten nicht mehr ausreichend konkurrieren und deshalb konnten wiederum alle Biotope neu erfüllt werden durch laufende, kletternde, springende, wühlende carni- und herbivore Typen.

Am eindringlichsten wird die Bedeutung dieses "Erfülltseins" des Raumes aber wohl durch die Tierwelt von ozeanischen Inseln verdeutlicht. So erreichten z.B. die Fringilliden-ähnlichen Vorfahren der heute endemischen Drepanididen die Hawai-Inseln, fanden hier fast alle Kleinvögelbiotope unbesetzt und spalteten in etwa 40 verschiedene Arten auf, wobei Fruchtfresser mit kurzen dicken Schnäbeln, Insektenfresser mit dünnen Schnäbeln, Gemischtfresser, Spezialisten mit langen Schnäbeln (die Insekten aus Baumspalten klaben) und schliesslich sogar Nektarsauger mit Röhrenzungen entstanden (vgl. Mordvilko, 1937). Es war also nicht ein Entfaltungsplan, der die Drepanididen-Formen entstehen liess, sondern es war die Fülle der neuen, von Konkurrenten nicht besetzten Biotope. Wären die Vorfahren der Drepanididen nicht nach der Hawaigruppe verschlagen worden, so hätten sie in ihrem Herkunftslande (wohl Südamerika) trotz steter Mutation nie ein solches explosives Aufblühen erleben können, sie wären Fringilliden geblieben, weil auf Insektennahrung oder auf Nektarsaugen spezialisierte Vogeltypen bereits in sehr grosser Zahl vorhanden waren.

Aber auch die zunehmende Spezialisierung, das Altern und Aussterben der

Formen lässt sich ausreichend durch Selektionsvorgänge deuten. Zunächst *bedeutet fast jede Selektion eine Verminderung der Euryökie*, d.h. eine Steigerung der Stenökie und der Spezialisierung (Hesse). Damit werden die Tierformen aber immer weniger befähigt, sich neuen Umweltsbedingungen anzupassen. Sehr stark stenöke Formen (d.h. lange selektionierte Formen) sind deshalb einem Aussterben am leichtesten ausgesetzt.

Wie wir sahen, führt die Selektion ja auch zur sukzessiven Grössensteigerung in den Stammesreihen. Nun ist es ganz selbstverständlich, dass kleine Formen sich wechselnden Umweltsbedingungen leicht anpassen können: ein kleines Säugetier kann zum Klettertier, Grabtier, Wühltier, etc. werden. Eine Riesenform vermag das nicht: sie wird deshalb einen Wechsel des Milieus oftmals nicht überdauern und aussterben. Nun kommt noch hinzu, dass mit der Grössenzunahme in den Stammesreihen infolge positiv heterogonen Wachstums vieler Organe auch immer gelegentlich Excessivbildungen auftreten *müssen* (Riesenhirsch z.B., vgl. Kap. V2), die in noch stärkerem Masse einer Umweltsänderung bzw. einer Verschärfung des Konkurrenzkampfes unterliegen. *Sowohl Entstehung zunehmender Stenökie als auch Herausbildung von Riesen- und Excessivformen sind also Folgen der stetigen Selektion und Ursachen des schliesslichen Aussterbens.* So ist der Verlauf einer Stammesreihe bis zum gewissen Grade "determiniert", doch braucht deshalb keine unbekannte innere Entwicklungstendenz, kein Sondertyp der Artbildung vorausgesetzt zu werden.

(5) Höherentwicklung

Stammesreihen, in denen ein neues Organ zum ersten Male gebildet wird, haben für die Entwicklungsgeschichte der Tierwelt stets eine besondere Bedeutung, und es ist immer wieder die Vermutung ausgesprochen worden, dass hier ein besonderes "Vervollkommnungsprinzip" wirksam sei. Wir müssen uns deshalb zum Schlusse noch die Frage vorlegen, ob der Artbildungstyp, der zu einer "Vervollkommnung", zu einer "Höherentwicklung" führt, ein prinzipiell anderer ist, als der, welcher nur zu Abwandlungen auf gleicher Entwicklungshöhe führt.

Da die Mutation richtungslos ist, und alle Allele ihr unterworfen sind, *stellt sich die Evolution zunächst als eine Erschöpfung aller morphologisch und physiologisch möglichen Gestalten dar, soweit sie tragbar sind*, d.h. nicht unmittelbar Fertilität und Vitalität (bzw. Konkurrenzfähigkeit) nachteilig verändern. So können sich also auch "sinnlose" Sonderheiten entwickeln, wie sie uns etwa in den fast ringförmigen Zähnen des Babirusa (positiv heterogones Wachstum) in vielen Excessivorganen und Luxusbildungen oder in der "unnötigen" Komplikation eines Entwicklungs-cyclus durch mehrere Zwischenwirte hindurch (*Fasciola hepatica*) entgegen treten.

Dieses Erschöpfen tragbarer Möglichkeiten kann man an fast allen Tiergruppen erläutern. Es möge genügen, drei beliebige Beispiele herauszugreifen. (1) *Excessives Wachstum einzelner Federn* ist fast im gesamten Gefieder der Vögel tragbar. Und so finden wir denn einzelne lange "Schmuckfedern" am Oberkopf (extrem beim Paradiesvogel *Pteridophora*), an den Kopfseiten (z.B. Trappe *Otts*), an Hals

und Brust (z.B. Truthuhn *Meleagris* und Paradiesvogel *Semioptera*), auf dem Rücken (Seidenreier *Garzetta*), an den Oberschwanzdecken (Pfau *Pavo*), am Schwanz (extrem bei Paradiesvögeln wie *Cicinnurus*, Paradiesfliegenfänger *Terpsiphone* u.a.) und selbst an den Schulterfedern (Mandarinente *Lampronessa*) und an den Handschwingen (Fahnen-Nachtschwalbe *Macrodipteryx*).—(2) Die *Brutpflegegewohnheiten der Vögel* erschöpfen ebenfalls fast alle hier denkbaren Möglichkeiten von der Ehelosigkeit (z.B. Kolibris) durch alle Zwischenstufen bis zur vollen Beteiligung beider Geschlechter an der gesamten Brutpflege, und andererseits bis zur alleinigen Brutpflege durch das ♂, bis zur sozialen Brutpflege der Pinguine und bis zum Brutparasitismus. Oder greifen wir hier nur den Nestbauinstinkt heraus: es baut entweder nur das ♀ (*Acrocephalus*), oder nur das ♂ (*Locustella luscinioides*), oder beide Geschlechter (*Troglodytes*) oder keiner (*Falco*).—(3) Die *Überwinterung von Insekten* kann praktisch in allen Stadien des Individualcyclus erfolgen, und auch hier sind alle Möglichkeiten verwirklicht. Schmetterlinge z.B. überwintern als Ei (*Lymantria*), als Larve (*Cosmotriche*), als Puppe (Spingiden) oder als Imago (*Vanessa*).

Unter den Möglichkeiten, welche richtungslose Mutation und Selektion verwirklichen, sind nun aber gelegentlich auch solche, die "zufällig" eine Zunahme der Komplikation oder eine gesteigerte Rationalisierung bedingen. In beiden Fällen ist ein Erhaltenbleiben möglich, im letzteren sogar zumeist wahrscheinlich. *Wenn so auf jeder Entwicklungsstufe eine Steigerung der Komplikation im Rahmen der normalen Mutation eintreten kann, so können wir auch in der gesamten "Höherentwicklung" nur die Erschöpfung einer möglichen Entwicklung sehen.* Natürlich soll dabei nicht verkannt werden, dass hier die Selektion immer wieder eine Steigerung der Differenzierung mit zunehmender Zentralisation ausgleichen muss (Franz, 1935), wie denn überhaupt die Frage der Anpassungsentstehung ein reines Selektionsproblem ist. Aber wir sehen jedenfalls, dass nicht irgendein immanenter Entfaltungsplan vorausgesetzt zu werden braucht und dass die besprochenen Typen von Rassen- und Artbildung auf der Grundlage von richtungsloser Mutation und Selektion zum Verständnis auch der grösseren Entwicklungslinien genügen.

Es sei dabei auch noch darauf hingewiesen, dass oft die wichtigsten Fortschritte zunächst nur als verhältnismässig wenig bedeutsame "Nebenanpassung" entstanden. So entwickelten sich z.B. die Lungen der Dipnoer wahrscheinlich ursprünglich nur als accessorische Organe zum besseren Überdauern der Trockenperioden.

VII. ZUSAMMENFASSUNG DER HAUPTSÄCHLICHSTEN GESICHTSPUNKTE

Rückblickend können wir feststellen, dass *richtungslose Mutation und Selektion generell als ausreichende Voraussetzungen für den Evolutionsprozess angesehen werden können* und dass gerade die so fruchtbare *ganzheitliche Betrachtungsweise keine Stütze für die Annahme eines übergeordneten immanenten Entfaltungsplanes bietet*. Es lässt sich jedoch *kein einheitliches "Modell" der Artbildung aufzeigen*, wie dies von manchen Genetikern gefordert wurde, sondern es liegen—den komplexen Daseins-

bedingungen der Organismen entsprechend—*verschiedene Typen der Rassen- und Artbildung* vor. Im einzelnen lassen sich dabei folgende Fälle unterscheiden.

(1) Richtungslose Mutation führt ohne erkennbare Selektion zu neuen Rassen und Arten. Es kann sich dabei auch um Abwandlung phylogenetisch wichtiger Organe (z.B. Genitaldifferenzen) handeln.

(2) Die Rassen- und Artbildung verläuft in bestimmten Richtungen, ohne dass eine Selektion erkennbar wäre.

(3) Die Selektion kann in bestimmten Richtungen verlaufen. Das führt z.B. bei klimatischer Auslese zu einer Merkmalsparallelität vieler Formen des gleichen Gebietes, die in einer Anzahl von biologischen Regeln formuliert werden kann.

(4) Ganzheitliche Formwandlungen lassen die Verursachung schwer erkennen, sind aber gleichfalls durch richtungslose Mutation und Selektion ausreichend zu erklären.

(a) Orthogenetische Entwicklungsreihen kommen zumeist durch stete Selektion grösserer individueller Varianten und durch die damit verbundenen Verschiebungen der Proportionen infolge heterogenen Wachstums einzelner Organe oder durch Orthoselektion zustande.

(b) Die zu wenig beachteten Kompensationserscheinungen, die in dieser Arbeit mit neuen Beispielen genauer belegt wurden, vermögen nicht nur ganzheitliche Formwandlungen zu erklären, sondern sind auch geeignet, den Vorgang der Reduktion und Rudimentation verständlich zu machen.

(c) Auch Umkonstruktionen im Sinne Bökers können ohne ein aktives Reagieren der Erbmasse auf Umweltsänderungen gedeutet werden.

(d) Ganz allgemein ist die pleiotrope Wirkung der meisten (aller?) Gene stärker in Rechnung zu stellen. Besonders gilt das auch für systematische Studien.

(5) Die erkannten paläontologischen Gesetzmässigkeiten der Evolution können ohne Annahme eines inneren Entfaltungstriebes verständlich gemacht werden.

(a) Eine Irreversibilität der Entwicklung ist durch Nachweis von Rückmutationen theoretisch widerlegt, besteht aber de facto in zahlreichen Fällen, weil die komplexen Bedingungen eine Wiederkehr der völlig gleichen Situation für Mutation und Selektion praktisch unmöglich machen.

(b) Orthogenetische Reihen wurden bereits in dem Kapitel über ganzheitliche Formwandlungen besprochen.

(c) Explosive Formaufspaltung und anschliessendes Nachlassen der Artbildung ist wegen der Stetigkeit der Mutation nicht auf wechselnde Mutationsraten, sondern auf zunehmende Erfüllung der verfügbaren Biotope mit konkurrierenden Formen zurückzuführen. Altern und Aussterben der Stammesreihen ist durch Zunahme der Stenökie und Spezialisierung infolge Selektion, durch selektiv bedingte Grössenzunahme und durch damit verbundene Entstehung von Excessivorganen zu deuten.

(d) Auch der Vervollkommenung und Höherentwicklung der Organismen liegt kein unbekanntes Entwicklungsgesetz zugrunde. Es handelt sich vielmehr nur um ein Erschöpfen der tragbaren Möglichkeiten, zu denen auch die Zunahme der Komplikation und der Rationalisierung zu rechnen ist.

VIII. SUMMARY

Undirected mutation and natural selection may be regarded as sufficient premises for evolution. In view of the manifold effects of mutations there is no justification in assuming an inherent process of unfolding. There is no simple scheme of species formation, as has been assumed by some geneticists, but there exist various types of race and species development corresponding to the complex conditions of existence of the different organisms. The following cases can be distinguished:

1. Undirected mutation leading to new races and species, without recognizable selection (e.g. *Rhipidura flabellifera*). Alterations of phylogenetically important organs, for example, genital organs, may be involved.

2. Race and species formation taking place in definite directions, without selection being recognizable (e.g. snails of the *Murella* group).

3. Selection occurring in definite directions. Through selection by climatic factors, this may lead to the parallel evolution of characters in different forms inhabiting the same region, which can be defined by various biological rules (e.g. geographical races of *Acrocephalus arundinaceus*).

4. The causes of transformations of the body as a whole cannot easily be recognized, but these are likewise explicable by undirected mutation and selection:

- (a) Orthogenetic series arise mostly through continual selection of the larger individual variants and the resulting changes of proportions due to the heterogonic growth of single organs, or through orthoselection (e.g. the ancestral series of *Equus*).

- (b) Insufficient attention has hitherto been paid to phenomena of compensation of growing body material. In this article they have been considered in greater detail (e.g. spines of the fresh-water gastropod *Melania*). They can explain not only transformations of the body as a whole, but also reductions and the formation of rudimentary structures.

- (c) Alterations of anatomical structure can be understood without an active reaction of the genes to environmental changes (e.g. skulls of *Potamochoerus*, *Hylochoerus*, *Phacochoerus*).

- (d) More emphasis should be placed on the pleiotropic action of most, or perhaps all, genes, i.e. the alteration of several characters by one mutation. This is particularly important in systematic studies (e.g. body size, tail, ears and feet of Muridae).

5. The evolutionary phenomena recognized in palaeontology can be understood without the assumption of any inner unfolding impulse:

- (a) Although the irreversibility of evolution has been demonstrated in fact in numerous cases, it has been disproved theoretically by the discovery of back-mutations. The actual irreversibility is due to the complex conditions of life which render a return of exactly the same situations for mutation and selection practically impossible.

- (b) Orthogenetic series have been dealt with under 4 (a).

- (c) Explosive development of new forms and later diminution of species formation should not be ascribed to varying rates of mutation, since mutation is steady, but to increasing occupation of the available biotopes with competing forms. Ageing and extinction of evolutionary series is to be explained by an increased restriction to narrow habitats and specialization due to selection, by selectively caused increase in size, and by the consequent development of monstrous organs.

- (d) Even the perfection and the higher development of organisms is due to no unknown law of development, but merely to the fulfilment of the available possibilities, including increase in complication and physiological amelioration and simplification.

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CHEMISTRY OF THE PLANT VIRUSES

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(Received September 15, 1938)

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I. INTRODUCTION

IN 1935 Stanley obtained from Turkish tobacco plants infected with tobacco-mosaic disease a semi-crystalline protein which when rubbed into the leaves of normal plants was capable of transmitting the disease. The purity of Stanley's protein was at once suspect, there being a body of opinion in favour of the view that the disease agent is not the protein but an impurity difficult to separate from it. Unfortunately, no unequivocal proof of the identity of protein and infective agent is possible, since there is no absolute criterion of the purity of a protein. However, a number of facts, mainly negative in character, have been discovered which collectively create a convincing case for the specific infective role of the protein. The most important of these is undoubtedly the fact that many attempts which have been made to separate by chemical or physical means an infective agent from the protein have been uniformly unsuccessful.

In the few years following Stanley's pioneer discovery a number of plant virus diseases were investigated by Stanley and collaborators in America and in this country, notably by Bawden and Pirie. In most of these diseases the specific proteins isolated are very similar in general physical and chemical properties to each other and to the tobacco mosaic protein. They are similar in elementary composition, and in molecular size and shape, and belong to the class of nucleo-proteins. Differences of a finer nature have been discovered, notably in X-ray reflexion spacings, salt precipitation and immunological reactions. One recently discovered virus protein, however, namely, that causing Bushy stunt disease, distinguishes itself fundamentally from the others by gross differences in the size and shape of the molecule and in a much higher content of nucleic acid. It appears, therefore, that a number of proteins not necessarily similar in chemical and physical properties exist which have a fundamental significance in plant pathology.

In the cognate field in animal pathology opinion has generally favoured the view that the virus agents are similar in nature to very small bacteria. The arguments which have been deduced for this view are based mainly on similarities of general biological behaviour and only to an insufficient extent on the physical properties of the virus particles themselves. One of the main arguments in the former category is that viruses and bacteria show a similar ability to multiply in association with living tissue. With the discovery of the plant-virus proteins it appears that this argument is weakened if not invalidated. A few milligrams of Stanley's protein rubbed into the leaves of a batch of tobacco plants will result in a few weeks in a yield of several grams of protein from the infected plants. This unequivocal new formation of foreign protein in contact with living cells must be accepted as a fact of great potential significance. The appearance of new protein molecules has generally been explained hitherto as an enzymic synthesis from free polypeptide units of smaller size. One would expect to find at some stage of this process evidence of the presence of proteoses and peptones such as are produced in the digestion or hydrolysis of protein. Unfortunately, there is a signal absence of material of this nature in the crude plant extract which might be associated with the synthesis of virus protein. It is not impossible, however, that plant virus molecules may multiply by a process of binary fission in the manner characteristic of bacteria and chromosomes. The acceptance of this view also has its difficulties, since it implies that at some stage in the process parent and daughter molecules should be present. The extreme homogeneity in size which virus molecules show in the ultracentrifuge must mean either that these molecules do not grow and divide in the manner suggested or that there is a rapid reproductive period followed by a longer quiescent phase in which the crude plant extract will only show the presence of forms of uniform size. Such a cycle is similar to that characteristic of nuclear division in the cell.

The view may be suggested that the plant viruses, and probably the smaller animal ones, represent the end of an evolutionary line of development in which high immunological specificity and physiological adaptability are incorporated in the chemical structure of large independent molecules. An alternative and more successful line of evolution in which biological complexity and adaptability are achieved by enclosing smaller protein molecules in solution inside a semi-permeable membrane has given rise to the bacteria. A suggestive analogy to this virus-bacterium relationship is to be found in the respiratory proteins. Here, nature in the first instance elaborated circulating protein molecules which attain large and complex forms in the haemocyanins of the invertebrates. Indeed, these haemocyanins bear a closer resemblance to the virus proteins in molecular size and homogeneity than do any other members of the protein kingdom. The erythrocyte, consisting as it does of a semi-permeable membrane enclosing a solution of low molecular protein, appears to develop independently and to adapt itself with greater success to the respiratory requirements of the higher animals. It may yet prove that there are other instances in nature of the localization and development of specialized functions in independent cellular and macromolecular forms. The cellular kingdom has been

thoroughly investigated, especially in the higher animals, but the investigation of heavy proteins in the lower forms of life is still in its infancy. The discovery of the plant-virus proteins is thus rich in implications for biology as a whole and may prove to have a far-reaching influence on our ideas of the constitution of the smaller forms of life.

II. PREPARATION

The first substance of this group to be isolated and characterized as a disease-producing agent was the tobacco mosaic virus. This was first isolated from the Turkish tobacco plant by Stanley (1935) and subsequently obtained by him also from infected tomato plants. Later, Stanley announced (1937*a*) the isolation of a protein from plants infected with aucuba mosaic, a disease with the same host distribution as the common tobacco mosaic but quite different in its symptomatology. Shortly afterwards Bawden *et al.* (1936) confirmed Stanley's investigations and extended them to include a third protein, that causing the enation variety of mosaic disease. Stanley & Wyckoff in 1937 prepared an unstable protein which is responsible for tobacco ring spot disease, and in the same year Bawden & Pirie (1937*a*) described the isolation in pure form of the cucumber viruses 3 and 4 which do not affect plants susceptible to tobacco mosaic. These authors also obtained (1938*a*) pure preparations of the potato "X" virus (or latent potato mosaic virus) which is a much less stable virus generally than the tobacco mosaic virus. Finally, Bawden & Pirie crystallized the virus of Bushy stunt (1938*b*), which has many distinctive features and appears to be in many respects the most clearly defined plant virus so far discovered.

Normal healthy plant sap, obtained by mincing either the whole plant or the leaves and expressing the juice, contains between 0.1 and 0.2 g. of soluble protein per litre of sap. In plants affected by the virus diseases the sap protein is often increased markedly, up to 0.5 g. per litre in the case of potato virus "X" and to as much as 2 g. per litre in severe infections with tobacco mosaic. In other cases, such as tobacco ring spot, little if any increase in the sap protein has been found.

Three general methods are in use for obtaining the viruses from the sap. Precipitation with less-than-half-saturated ammonium sulphate was the method originally used by Stanley to isolate the tobacco mosaic virus. In general a concentration of this salt varying between 10 and 20 g. per 100 ml. of solution precipitates any of the viruses completely from neutral solution. In similar conditions most of the protein of the normal sap is not precipitated. An alternative method of precipitation is to acidify with hydrochloric, sulphuric or acetic acids preferably in the presence of moderate concentrations of salt. The hydrogen-ion concentration at which maximum precipitation occurs varies from virus to virus, and is naturally also dependent on the salt concentration. Thus in the same conditions of salt concentration the maximum precipitation of tobacco mosaic virus and cucumber virus occurs at pH 3.4 and 4.8 respectively. In the absence of salt these pH values become 4.2 and 5.5 respectively. The precipitates so obtained from

solutions of the anisotropic viruses are characterized by a satin-like sheen. The precipitate from Bushy stunt, which is isotropic, does not show this sheen.

A third method which has proved very useful where only a small quantity of virus is present in the sap consists in centrifugal deposition. As the heavy protein concentrates at the bottom of the cell the viscosity of the bottom layer increases until a rigid gel or so-called "pellet" is formed. The gel also contains an amount of inactive non-sedimenting protein proportional to the volume of fluid in the gel. The pellet is therefore redispersed in water, the solution clarified at low speeds and another centrifugal deposition effected. In some circumstances the method may not be effective, especially where the healthy plant sap contains proteins of the same order of molecular size as the viruses. Loring *et al.* (1938) have shown that such heavy proteins are to be found in the sap of the healthy broad bean and pea, and Price & Wyckoff (1938) have demonstrated their presence in normal cucumber sap. Bawden & Pirie (1938*d*) have also found them in normal tobacco and tomato saps and have shown that they denature and precipitate in a few days at room temperature.

There is some evidence also that normal plant proteins and other substances are precipitated along with the viruses. The virus from a heavily pigmented sap is still coloured after many precipitations. Chester (1936) has shown that virus precipitates contain normal tobacco protein which is distinguishable from the virus by being more strongly anaphylactogenic. Further evidence relating to the purity of a virus preparation is obtainable from observations on the behaviour of a strong solution of the protein.

Bawden & Pirie (1937*b*) have shown that solutions containing more than about 4% of tobacco mosaic virus separate on standing into two layers, an upper layer which is less concentrated than 2% and a lower layer which is liquid crystalline. If the upper layer is separated and concentrated *in vacuo* it will again separate into two layers. Similarly, dilution of the bottom layer leads to the separation of a new more dilute bottom layer. The bottom layer contains less impurities than the top layer, and a process of repeated dilution and separation of bottom layers may be used as a means of virus purification. At concentrations below about 2% solutions of the virus proteins cease to separate into layers. The critical concentration at which layering occurs and the difference in concentration between the layers is influenced by the amount of impurities present. A virus preparation which will not form a spontaneously birefringent lower layer at concentrations below about 4% is always capable of further purification.

There are a number of procedures which may be used as adjuncts to the precipitation or centrifugation methods in order to prepare a plant virus which is colourless, not anaphylactogenic, and spontaneously birefringent at low concentrations. A first and most important step is to choose a favourable starting material. The sap from young actively growing plants which have been infected for 3-4 weeks contains large amounts of virus and much less pigment than that from older plants. A preliminary clarification of the sap is effected by centrifugation at low speeds, standing overnight, or filtration through celite or kieselguhr. The clear sap

may then be precipitated with an equal volume of alcohol and the virus extracted from the precipitate with water, whereby an insoluble coloured residue, mainly carbohydrate in nature, is obtained. Lead acetate was used in earlier work by Stanley instead of alcohol but was discarded when it became evident that it caused considerable inactivation of the virus. The alcohol precipitation is also to a large extent now replaced by more effective procedures. One such, due to Bawden & Pirie (1937*b*), consists in heating the crude sap to 60–70° C., when a precipitate containing much plant impurity and pigment, but having little virus activity, is obtained. The same authors also recommend incubation with 0.2% commercial trypsin for 10–30 hr. at 37° C. and pH 7–8. The enzyme has little effect on most of the viruses, and the treatment invariably yields a product which becomes colourless after two or three further precipitations with acid and dilute ammonium sulphate. The potato "X" virus is an exception in being sensitive to the action of trypsin although not so sensitive as the impurities associated with it. In this case 0.05% pancreatin for 90 min. is recommended, a reduction to two-thirds of the original virus activity resulting from the treatment. The method of successive dilution of bottom layers may be used with advantage as a final step in the preparation of pure virus. The layering phenomenon is of course primarily the result of the anisotropy of the protein molecules, and the method cannot therefore be applied to an isotropic protein such as the virus of Bushy stunt.

III. ELEMENTARY COMPOSITION

The figures in Table I are taken from Stanley (1936, 1937*a*) and Loring & Stanley (1937) and represent percentages of the ash-free dry weight

Table I

Virus	Host plant	C	H	N Dumas	Cl	P	S	Ash
Tobacco mosaic 1	Tobacco	52.00	7.00	16.09	—	—	—	1.51
		52.38	6.94	16.17	—	—	—	1.77
Tobacco mosaic 2	Tobacco	53.14	6.90	16.36	0.50	0.00	0.00	1.49
		53.25	6.99	16.25	0.52	0.00	0.00	1.30
Tobacco mosaic 3	Tobacco	50.74	7.56	16.56	—	0.01	—	0.53
Tobacco mosaic 5	Tomato	50.93	7.58	16.70	—	0.21	—	1.29
Tobacco mosaic 2	Tomato	51.57	6.91	16.20	—	—	—	1.64
		51.37	6.95	16.35	—	—	—	1.43
Aucuba mosaic 1	Tobacco	50.46	6.88	16.52	0.14	—	—	0.11
		50.30	6.94	16.59	0.12	0.51	0.24	0.17
Aucuba mosaic 2	Tobacco	49.07	6.64	16.67	1.35	—	—	0.19
		49.33	6.68	16.79	1.36	—	—	0.11

Tobacco mosaic preparation 1 was dialysed at pH 4.5 for 4 days, and 2 was dialysed at pH 3.25 for 8 days. Both were subsequently precipitated with acetone and dried over P_2O_5 *in vacuo* at 60° C.

Tobacco mosaic preparations 3 and 5 were dialysed against distilled water at pH 7 for 7 days, then precipitated and washed with acetone. Preparation 2 from the tomato plant was treated in the same way but was also dialysed for 2 days at pH 4.

Aucuba mosaic preparations were dialysed against water at pH 7 for 1 day and then against dilute HCl at pH 3.7 for 4 days. 1 was precipitated with acetone and dried over P_2O_5 *in vacuo* at 60° C. 2 was precipitated with hot 2.5% trichloroacetic acid, then twice with water and dried over P_2O_5 *in vacuo* at 60° C.

The figures in Table II for dried neutral solutions are taken from Bawden & Pirie and represent the limits of their analytical results expressed as percentages of the dry weight.

Table II

Virus	C	H	N	S	P	Ash	Carbohydrate
Tobacco mosaic*	49.3	7.2	14.4	0.24	0.45	1.5	2.5
Cucumber 3 and 4†	50.0	7.4	16.6	0.59	0.55	3.0	
	50.0	7.1	15.3	0.00	0.55	1.0	2.2
	51.0	7.6	15.8	0.60	0.60	2.0	2.6
Potato "X"‡	47.7	7.1	15.7		0.4	2.0	2.5
	49.5	7.7	17.0		0.5	2.5	3.0
Bushy Stunt§	47	7.3	16		1.3		6.0

* Bawden & Pirie (1937*b*).

† Bawden & Pirie (1938*a*).

‡ Bawden & Pirie (1937*c*).

§ Bawden & Pirie (1938*b*).

There is good agreement among the different observers except in relation to phosphorus and carbohydrate, in which connexion there appears to be an important difference of opinion. Bawden *et al.* (1936) first drew attention to the fact that tobacco mosaic virus contains phosphorus and carbohydrate which can be isolated as nucleic acid. Stanley (1937*a*) states that it is possible to remove this nucleic acid by dialysis at pH 8 or 9 and to obtain a phosphorus-free protein possessing virus activity. He concludes that the nucleic acid is not an integral part of the molecule. This conclusion is not shared by Bawden & Pirie who write (1937*b*): "the phosphorus and carbohydrate contents of our preparations are very constant. They are unaffected either by prolonged dialysis in cellophane tubes against dilute acid or alkali, or by reprecipitation 10 times with either acid or quarter saturated ammonium sulphate solution. From precipitates with their antisera, and with papain and clupein sulphate, the viruses have been recovered with their full activity and with their phosphorus content unaltered, and incubation with trypsin preparations rich in nuclease has no effect on the phosphorus content. All the treatments which we have tried which in no way inactivate the virus preparations leave the phosphorus content unaltered." These authors are therefore convinced that the nucleic acid is firmly bound to the protein and that the plant viruses are true nucleo-proteins. Best (1937) has found 0.52% phosphorus in his tobacco-mosaic virus preparations and agrees with Bawden & Pirie that this is bound to the preparation and inseparable from it without loss of activity. Loring (1938*a*) has also confirmed the presence of nucleic acid which he has prepared in yields of about 5% by alkaline hydrolysis with sodium hydroxide at 0° C. or by treatment with glacial acetic acid. The product resembles yeast nucleic acid, and the presence of guanine, adenine, uracil and cytosine has been demonstrated. Loring also agrees that the nucleic acid is essential to virus activity.

IV. SEDIMENTATION BEHAVIOUR

The first study of the behaviour of these proteins in centrifugal fields was made by Eriksson-Quensel & Svedberg (1936) on a preparation of tobacco-mosaic virus supplied by Stanley. This proved to be inhomogeneous both before and after re-

peated "crystallizations". The mean sedimentation constant was 235 in the range pH 6-8. These authors subsequently examined further preparations of the virus grown on tobacco and tomato plants and found these to be more homogeneous than the earlier preparation. The sedimentation constants were 190 and 197 for the proteins from the tobacco plant and 202 for that from the tomato plant. At pH 9.8 the tomato protein had split into components of 185 and 125.

Wyckoff *et al.* (1937) found with the aid of the air-driven ultracentrifuge that while most of their tobacco-mosaic preparations were inhomogeneous a few gave rise to sharp boundaries (Fig. 1). For the particular protein concentration which they used, viz. 1.6 mg. per ml., they obtained sedimentation constants varying between 170 and 250. The range of variation is reduced to 175-195 if one considers only preparations from mature tobacco plants. There is some evidence that the proteins from young plants have higher sedimentation constants than those from older plants. Tobacco-mosaic proteins grown on tomato and phlox plants have



Fig. 1. An absorption run on a solution of the tobacco mosaic virus protein in 0.1 *M* phosphate buffer pH 7. (Wyckoff, 1937*a*.)

sedimentation constants in the same range as those from tobacco plants. To this extent virus proteins appear to be the same whatever host plants they come from. On the other hand, there is evidence that different strains of a virus correspond to physically distinguishable proteins. The protein of the aucuba strain of tobacco mosaic has a sedimentation constant some 20% higher than that of the ordinary variety prepared under comparable conditions (Stanley, 1937*a*). Cucumber 3 and 4 viruses, on the other hand, have closely agreeing values, viz. 172 and 175. The lowest sedimentation constants recorded belong to tobacco ring spot virus at 115 and latent potato mosaic or "X" virus at 113. Preparations of Bushy stunt virus made by Bawden & Pirie were exceptionally homogeneous (Fig. 2) and had a mean sedimentation constant of 146.

Table III. *Sedimentation constants of the plant viruses*

Virus	Host	S_w , 20° C.	Observer
Tobacco mosaic	Tobacco	190, 197, 235	Eriksson-Quensel & Svedberg (1936)
" "	Mature tobacco	175-195	Wyckoff <i>et al.</i> (1937)
" "	Tomato	173, 207	Wyckoff <i>et al.</i> (1937)
" "	Phlox	174	Wyckoff <i>et al.</i> (1937)
Aucuba mosaic	Mature tobacco	220-240	Wyckoff <i>et al.</i> (1937)
Latent potato mosaic	Tobacco	113*	Loring & Wyckoff (1937)
Cucumber 3 and 4	Cucumber	172, 175	Price & Wyckoff (1938)
Ring spot	Tobacco	115	Stanley & Wyckoff (1937)
Bushy stunt	Tomato	146	McFarlane & Kekwick (1938)

* Small amount of second component for which S_w , 20° C. = 131.

On more than one occasion it has been found that a preparation of a virus has exhibited two boundaries instead of one. Wyckoff *et al.* (1937) report such a case in which the sedimentation constants of the components were 184 and 217. Wyckoff (1937*a*) produces evidence that these double boundaries result from the action of salts on a homogeneous protein. When virus which has been centrifuged directly from the sap is resuspended in distilled water a single boundary is obtained. Addition of 0.1 *M* phosphate to this protein solution at neutral or weakly alkaline reactions produces two boundaries.

The data in Table III refer to dilute solutions of the viruses. We have already discussed the formation of top and bottom layers in concentrated solutions of the anisotropic viruses. The behaviour of samples of these two layers on centrifuging is different and has been studied in detail by Bawden & Pirie. When the top-layer solution is centrifuged in fields of approximately 16,000 times gravity a viscous jelly is deposited. After 1-3 hr. most of the virus is deposited from such a

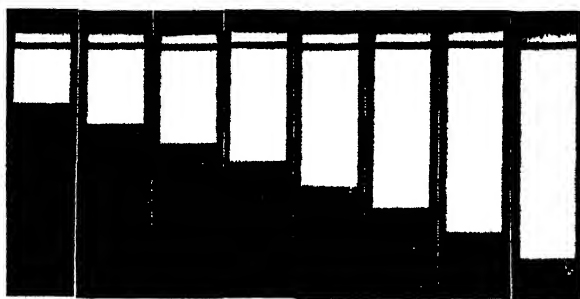


Fig. 2. Sedimentation of the virus protein of Bushy stunt in 0.02 *M* acetate buffer at pH 3.4. (McFarlane & Kekwick, 1938.)

neutral solution leaving a dilute solution of virus immediately above the jelly and a layer of water at the meniscus. The interesting fact emerges that within certain limits the time of centrifuging does not affect the concentration of the jelly but only the total amount of jelly formed. Bottom-layer solution, on the other hand, deposits a jelly which may have less than half the solid content of that sedimented from top-layer solution of the same concentration. There is no sharp interface in this case between the jelly and the fluid above such as exists in the case of centrifuged top layer. These relationships enable one by varying the concentration of the centrifuged fluid to obtain stable jellies with very similar physical appearances, but with solid contents ranging from 10 to 35 %. These jellies are, of course, all highly birefringent.

V. pH STABILITY

An investigation of the pH stability range of the plant viruses is hampered by the fact that with the exception of Bushy stunt virus they all show somewhere between pH 2 and 6 a zone of minimum solubility, and anomalous sedimentation. On both sides of this zone homogeneous proteins have been found. Below pH 1.5

denaturation is rapid, the preparations becoming completely inhomogeneous (Fig. 3) and nucleic acid being split off. Above pH 10 the proteins are also in general rapidly inactivated, and the centrifuge picture frequently shows two or more components. The occurrence of these components may not, however, be due entirely to the hydrogen-ion concentration in view of the effect of salts to which we have already referred. Further investigations are required to differentiate between these two influences, which are no doubt mutually dependent.

The pH at which acid or alkaline denaturation first becomes apparent varies from protein to protein. In the case of the ordinary tobacco mosaic protein the insolubility zone extends from pH 2.8 to 5. Below pH 2.8 the protein is homogen-

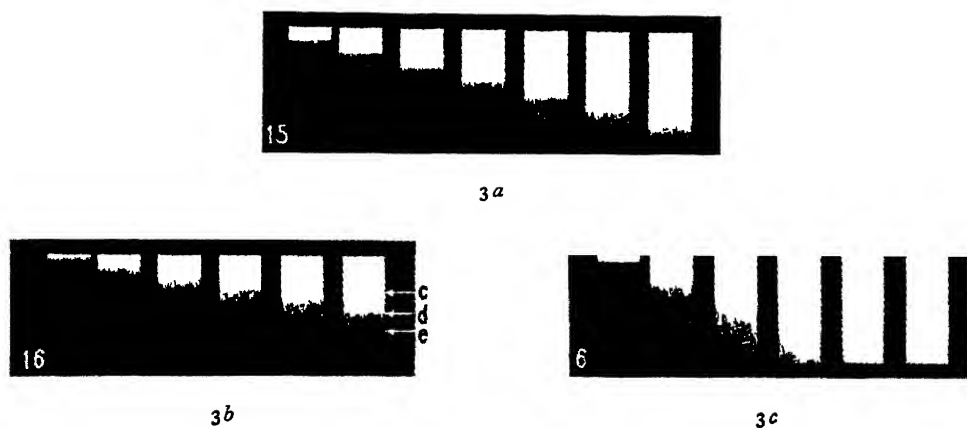


Fig. 3. *a*, tobacco mosaic virus protein at pH 10 immediately after mixing. *b*, the same after 1 day. *c*, tobacco mosaic virus protein after 65 days at pH 1.8. (Wyckoff, 1937*b*.)

eous and even shows no immediate change after adjusting to pH 1.8 (Wyckoff, 1937*b*). After 15 hr. at this pH , however, it is completely inhomogeneous. On the alkaline side tobacco mosaic protein shows no instantaneous change at pH 10. After 2 days at pH 9, however, two boundaries are present in addition to quantities of protein of much smaller molecular weight. In contrast to this tobacco ring spot protein is completely denatured after 1 hr. at pH 3 and only slightly affected after 1 hr. at pH 9.6. Inside the insolubility zone the anisotropic proteins aggregate progressively the viscosity of the solution increasing rapidly, accompanied by an increasing opalescence. A point of maximum precipitation is ultimately reached, the pH depending as already stated on the salt concentration. When the protein solution is adjusted to a pH close to but not coincident with the zone of actual precipitation the virus can be deposited by fields of a few thousand times gravity using an ordinary laboratory centrifuge. The virus of Bushy stunt differs from the others in showing no zone of actual or incipient precipitation. Between pH 2.4 and 8.7 it is completely homogeneous for long periods and shows no evidence of aggregation. After 1 hr. at pH 10 or 1.3 two boundaries are present, the sedimentation constants being 112 and 135 in both cases (McFarlane & Kekwick, 1938).

VI. ELECTROPHORETIC BEHAVIOUR

Even the very inhomogeneous preparation of tobacco mosaic protein investigated in the centrifuge by Eriksson-Quensel & Svedberg proved to be electrochemically homogeneous to a high degree (Fig. 4). They examined the material in the range pH 3.63–4.95, in part of which the preparation was aggregated. Nevertheless consistent measurements were obtained which gave pH 3.49 as the isoelectric point in 0.02 *M* sodium acetate buffer, and 12.3×10^{-5} as the slope, $du/d(pH_0)$, of the pH -mobility curve. The only other virus for which complete electrophoresis data is available is the virus of Bushy stunt. This also migrates in an entirely homogeneous manner in 0.02 *M* acetate buffers over the range pH 3.8–6.2 (McFarlane & Kekwick, 1938). It gives a well-defined isoelectric point at pH 4.11, and the slope of the pH -mobility curve is 5×10^{-5} . Loring, quoted by Stanley (1937*a*), found the

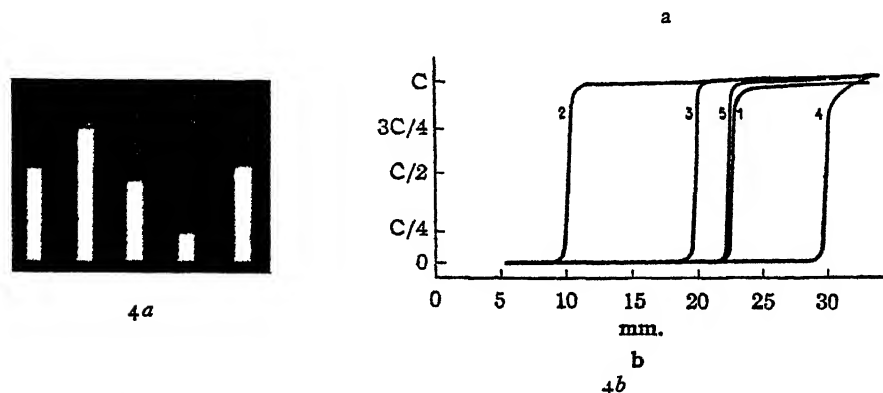


Fig. 4*a, b*. Electrophoresis pictures (*a*) and the corresponding concentration curves (*b*) for tobacco mosaic virus protein. (Eriksson-Quensel & Svedberg, 1936.)

isoelectric point of aucuba mosaic protein at pH 3.7 in conditions which are not stated. In a subsequent paper Loring & Stanley (1937) give the results of some electrophoresis experiments on suspensions of virus "crystals" using the Northrop-Kunitz apparatus. Below pH 3.2 their preparations of tobacco mosaic protein from tobacco and tomato plants were positively charged, and above 3.35 negatively charged.

VII. ANISOTROPY OF THE PLANT VIRUSES

No property of these interesting proteins has been a greater source of popular interest or a stronger inspiration to their further investigation than the optical behaviour resulting from the asymmetry of their molecules. Takahashi & Rawlins (1933) first observed birefringence of flow in clarified infective sap before the existence of the virus proteins was known, and attributed it to the presence of asymmetrical particles in the sap. In 1936 Bawden *et al.* gave the first complete account of this phenomenon in relation to the tobacco mosaic protein. They demonstrated the spontaneous birefringence of lower layer solutions and the optical anisotropy which can be induced by mechanical agitation in upper layer solutions. Their

solutions could be orientated by electric currents but not by magnetic fields of 6000 gauss.

Birefringence can be induced to a variable degree in solutions of all the plant viruses yet purified with the exception of the virus of Bushy stunt. Crude infective sap and impure virus solutions show less birefringence than purified preparations. The persistence of the effect is also much shorter. Lauffer & Stanley (1938) have discussed the probability that the birefringence of the viruses may be due to a "photoelastic" effect, but they ultimately dismiss this idea, since birefringence of "photoelastic" origin should cease immediately the strain or shearing force is removed.

Bawden *et al.* also drew attention to the fact that when lower layer virus is dispersed mechanically in upper layer the gel is fragmented into birefringent spindle-shaped bodies (Fig. 6). These bodies have an approximately constant meridional curvature, the larger bodies approaching a spherical shape and the smaller ones being almost linear. Such tactoid bodies are the result of the competing tendencies of surface tension on the one hand, which tends to form small spheres, and mutual orientation of the molecules on the other hand, tending to produce rods (Bernal 1938*a*). This phenomenon as well as the birefringence is best explained by the assumption of rod-shaped molecules, and this view is now widely accepted. The length of the molecules is not yet determined with any accuracy, and indeed there is not sufficient evidence to show that the molecular units of a particular preparation are all of the same length.

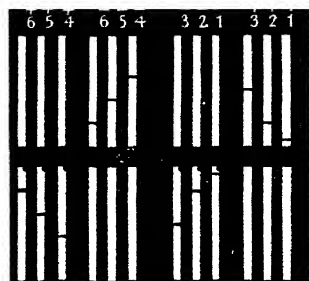


Fig. 5. Electrophoretic migration of Bushy stunt protein 0.5% in 0.02*M* acetate buffer pH 6.2. Photograph by Schlieren method. (McFarlane & Kekwick, 1938.)

VIII. VIRUS "CRYSTALS" AND "GELS"

Wyckoff & Corey (1936) investigated the X-ray diffraction pattern given by Stanley's tobacco mosaic virus "crystals". They recorded a number of sharp reflexions corresponding to spacings between 3 and 80 Å., and concluded that they were dealing with "true crystals composed of large molecules". Bernal *et al.* confirmed their measurements but found additional small angle reflexions which led them to another conclusion.

Fig. 7 shows the appearance of aucuba mosaic virus "crystals", and it will be seen that the definition of these is on the whole of a low order. They appear to have as great a similarity to fibres as to crystals. Wyckoff & Corey investigated these in the form of a paste. Bernal and collaborators, on the other hand, investigated the patterns obtained from the virus in three different states, viz. in the form of top-layer solution orientated in glass capillaries; in the form of a 50% solution, which they call "wet gel"; and in the form of a translucent semi-dry film obtained when the wet gel is allowed to dry in air. The most striking feature of their results is that

so far as the large angle scattering is concerned the pattern given by all three states is essentially the same and in agreement with the pattern obtained by Wyckoff & Corey. The only regularities which could conceivably arise from the same molecules in such a variety of physical states must arise inside the molecules themselves. This is entirely reasonable, since the pattern in question has about the same complexity as a characteristic protein intramolecular pattern, such as that produced by feather keratin, with a repeat unit in the fibre direction of $3 \times 22.2 \pm 0.2 \text{ \AA}$. There is no fundamental difference which X-rays can reveal between the so-called crystals and the wet gel, and Bernal (1938*b*) expresses the opinion that the "crystals" originally described by Stanley are really microtactoids. As this author points out, the sole evidence of crystallinity is a two-dimensional regularity which is shared by the wet

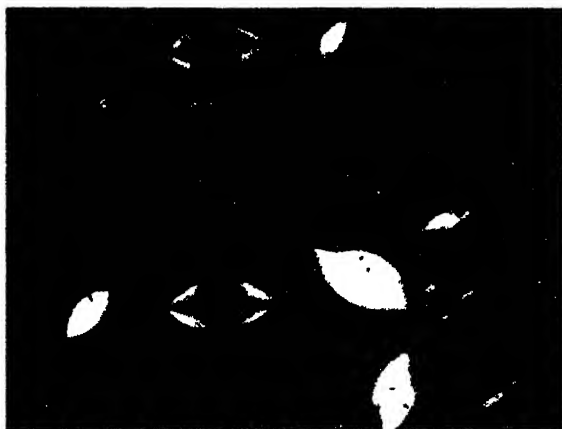


Fig. 6. Birefringent tactoids seen in a solution of purified tobacco mosaic protein. Photographed between crossed nicols, $\times 30$. (Bawden *et al.* 1936.)

gel and indeed by any form of natural fibre. In common with the latter tobacco mosaic virus "crystals" appear to have no determinate length; instead of having end-faces they tail off to a point at each end. There is, however, evidence that virus crystals which can be seen inside plant cells have well-defined end-faces as well as side-faces, and these of course deserve to be described as crystals as much as any other protein "crystals" do. Some influence appears to be lacking in our methods of artificial crystallization which is necessary to the completion of the crystal in the long axis. Probably this is merely a question of knowing the correct conditions. Nevertheless, to describe as crystals the two-dimensional fibres present in artificial preparations of the anisotropic viruses seems unwarranted and only likely to introduce further confusion into a term which is already in danger of becoming meaningless. It is of interest that the only isotropic member of the group, the virus of Bushy stunt, should form well-defined crystals belonging to the cubic system (Fig. 8).

From investigations of the small angle scattering of their preparations of tobacco mosaic virus Bernal *et al.* conclude that these patterns arise from intermolecular

regularities. There is a perfect hexagonal pattern common to wet and dry states, and the scale of which, unlike that of the large angle scattering, appears to vary quantitatively with the amount of water inserted between the particles. This pattern corresponds to an effective diameter of 152 \AA . and to a minimum cross-sectional area of $20,100 \text{ sq. \AA.}$ for the unit in the dry gel. The regularity is in one plane only. In a plane at right angles no regularity of any kind could be discovered, although any such less than 1200 \AA. would have been detected. The molecules therefore appear to be at least 10 times as long as they are broad. The whole picture is that of a bundle of parallel rods more or less closely packed together according to the amount of water present. As already stated, there is no evidence that these rods have the same length. Even if they have the same length the possibility exists that they may not lie in the fibre with their ends coplanar, but may overlap each other.

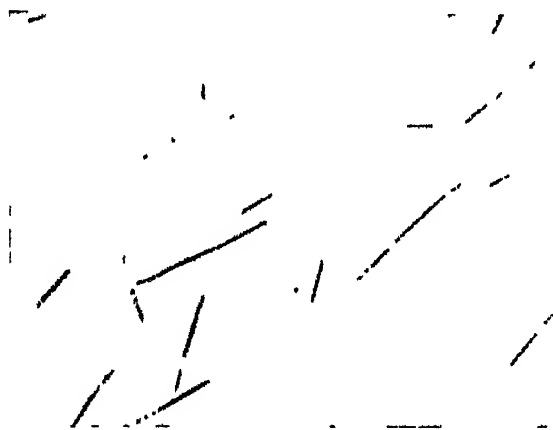


Fig. 7. "Crystalline" aucuba mosaic virus protein isolated from Turkish tobacco plants, $\times 393$. Photograph by J. A. Carlile. (Stanley, 1937*a*.)

IX. MOLECULAR DIMENSIONS

Any estimate of the molecular weights of the anisotropic viruses must depend on an estimate of the asymmetry of the molecules. The usual sedimentation-diffusion method of determining the dissymmetry constant is not applicable because of the very small diffusion which these large molecules show. Eriksson-Quensel & Svedberg obtained a mean molecular weight for tobacco mosaic protein of about 17 millions based on sedimentation equilibrium measurements for which a low accuracy is claimed. A similar figure is obtained from their sedimentation data if a dissymmetry constant of 1.3 is assumed.

Bawden & Pirie have attempted to apply Staudinger's theory of the free movement of rod-shaped molecules to tobacco mosaic virus. It is assumed that the volume of water in which the molecule will just rotate freely is that of a flat cylinder, the diameter of which is equal to the width of the molecule. This volume

can be obtained from the critical concentration for spontaneous birefringence. Using 152 Å. for the width of the molecule this method gives 14,000 Å. for the length. Staudinger is of opinion, however, that in the case of his linear polymers the solutions turn from the "sol" to the "gel" state when the volume available for each rod is much less than the volume of the corresponding flat cylinder. The value of 14,000 Å. for the length is therefore probably an overestimate rather than the opposite.

Kuhn (1933) has derived an expression relating the viscosity of a suspension of rods to the relative dimensions of the rods. Frampton & Neurath (1938) and Lauffer (1938*a*) both find that the viscosity of solutions of tobacco mosaic protein bears an almost linear relationship to concentration up to concentrations of 1%. From the value of the viscosity coefficient and Kuhn's equation these authors calculate that the length of the molecule is 35-40 times the width. According to equations derived by Perrin (1936) such a ratio should correspond to a dissymmetry constant of approximately 2.5, and from this value and sedimentation velocity data Lauffer calculates the molecular weight to be 42 millions. Alternatively, if the rod is cylindrical with a diameter of 152 Å. and a length 40 times this value then the volume and weight of a molecule may be calculated. Assuming a density of 1.37 this method gives approximately 90 millions as the molecular weight. This and Lauffer's estimate depend on a number of important assumptions, notably that Kuhn's equation is valid in the circumstances of the sedimentation experiment and that the virus protein has little or no water of hydration.

In the case of the Bushy stunt virus all the signs of molecular asymmetry are absent, and it is possible to determine the molecular weight with considerable accuracy. Sedimentation equilibrium determinations with fields of 150 times gravity have given a molecular weight of 7,600,000 (McFarlane & Kekwick, 1938). If the shape of the molecule is truly spherical the fractional force opposing sedimentation is given by Stokes's expression, viz. $6\pi\eta r \frac{dx}{dt}$, and the radius of the particle is given by

$$r^2 = \frac{9\eta S_{\pi, 20}}{2(\rho_v - \rho_s)},$$

where η is the viscosity of water at 20° C., and ρ_v and ρ_s are the densities of the protein and solvent respectively. Substituting $S_{\pi, 20} = 1.46 \times 10^{-13}$ in this equation, the radius of the molecule is 13.7 m μ and the molecular weight of such a sphere 8,800,000. The degree of agreement between the equilibrium and sedimentation values indicates that the molecule cannot depart to any great extent, if at all, from a symmetrical shape. If the molecular weight of the tobacco mosaic virus should be for any reason of the same order as that of Bushy stunt protein its molecular length would require to be approximately four times its breadth.

The partial specific volumes of the plant viruses have been determined by various workers. The value 0.646 obtained by Eriksson-Quensel & Svedberg for Stanley's early and very inhomogeneous preparation of the tobacco mosaic protein is unusually low and fails to agree with Bawden & Pirie's value of 0.73 for the same protein. McFarlane & Kekwick obtain 0.74 for the Bushy stunt protein.

X. SOME GENERAL PROPERTIES

There appear now to be no grounds for doubting that these protein molecules are themselves the cause of the disease which results from their introduction into the plant. Stanley (1937*b*) has made many attempts to separate a fraction of smaller size capable of causing the disease but without success. As we have already pointed out, the apparent crystallinity of these substances is a doubtful criterion of their purity, even in the case of bushy stunt. Indeed, there is some evidence in the case of this virus that preparations giving larger crystals (Fig. 8) contain more impurity than those giving smaller ones. In any case it is well known that protein crystals as a

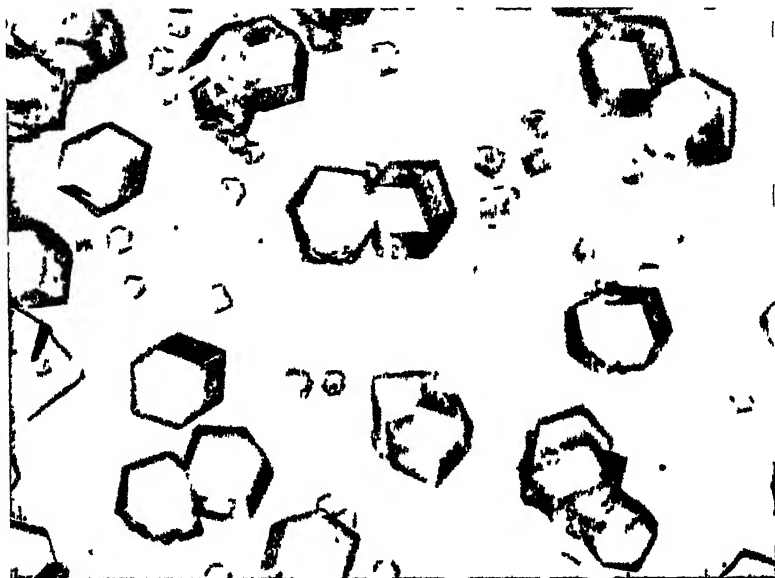


Fig. 8. Crystals of Bushy stunt virus protein. (Bawden & Pirie, 1938*c*.)

class are in equilibrium with constituents of the mother liquor other than the protein itself (Adair & Adair, 1936) and are to be regarded as more in the nature of solid solutions. Of greater importance in relation to the purity of the viruses is probably the fact that the scale of the intramolecular regularity which X-rays reveal in all oriented preparations is so small, in the region of 22 Å., that it permits of little else being present inside the molecule. The fact that this regularity is the same in the dry film and in the wet gel indicates that there cannot even be much more water in the molecule than is ordinarily associated with hydrated proteins.

There is some evidence, however, that some of the viruses exist in the sap in a smaller molecular form than in pure preparations. Infective sap shows much less birefringence than the purified virus obtained from it. When the purified virus is added to clarified sap the birefringence is greater than that due to the same amount of virus in the untreated sap. Even after one precipitation the anisotropy of flow is increased and even one centrifugal deposition and resuspension has the same

effect. Bawden & Pirie (1937*b*) deduce from these facts that a linear aggregation takes place, and in support of this they show that tobacco mosaic virus in the crude sap will readily pass through collodion filters which completely retain the purified preparation. On the other hand, Wyckoff *et al.* (1937) find very little difference in the sedimentation rate of crude and purified virus. It is possible that purely linear aggregation may have only an insignificant effect on the sedimentation constant owing to the relatively large increase in surface which accompanies a given increase in the weight of the molecule. Further investigation of this important point is clearly required. There is no question of aggregation in the case of Bushy stunt virus, since this requires approximately the same centrifugal force to deposit it from the crude sap and from purified solutions.

Plants inoculated with 10^{-9} g. of the viruses usually become infected, and infections have been known to follow inoculations of as little as 10^{-11} g.

The plant viruses rotate the plane of polarized light and have a region of specific light absorption in the ultra-violet in conformity with their protein constitution. It is difficult to measure anything corresponding to true solubility in these substances. In common with the proteins generally no solid phase separates from solutions as they are concentrated, but only gel formation occurs. In the presence of salts solid or quasi-solid virus protein is formed, but when this is separated by centrifugation in order to determine the amount of soluble protein in the supernatant it is found that the latter value is largely a function of the time of centrifugation. Loring & Stanley (1937) have obtained some figures using arbitrary conditions of centrifugation, but they do not claim that the results represent more than a rough estimate of relative solubility.

Some of the viruses preserve their characteristic properties and activity over periods of months or years in solution in the cold, being little affected by most enzymes and not susceptible to bacterial action. There are, however, few rigid rules of behaviour which one or other member of the group does not break. Thus potato "X" virus is susceptible to tryptic digestion. Whereas tobacco mosaic virus solutions are unaltered after long periods at room temperature, tobacco ring spot virus is partially inactivated after 1 day and completely inactivated after 6 days at room temperature. On heating to 70° C. for a few hours tobacco mosaic virus is unaltered, whereas potato "X" and tobacco ring spot viruses are inactivated in a few minutes. Heating neutral solutions to 90° C. coagulates tobacco mosaic virus, but no visible precipitate is produced in salt-free solutions of potato "X" virus even on boiling.

Purified solutions of tobacco mosaic virus may be partially dried over sodium sulphate or ammonium sulphate without loss of activity. Complete dehydration, whether in the frozen or unfrozen states, always results in a partial inactivation of the virus and in a marked reduction in the anisotropy of flow and in the ability to form liquid crystalline solutions.

Various chemical and physical agents which affect the viruses may be classified in two groups. In one which includes nitrous acid, formaldehyde, dilute hydrogen peroxide and X-rays, the general effect is to destroy the infectivity of the

viruses without altering their serological or optical behaviour. In the other group, which includes heating to 70° C. or over, and treatment with strong alcohol or acid, the effect is to destroy the serological and optical properties as well as the infectivity. Glacial acetic acid splits off the nucleic acid as an insoluble residue while effecting solution of the protein part of the molecule. Urea which exerts a strong denaturing action on most proteins appears to have only a slight influence on tobacco mosaic protein, solutions of which retaining their ability to give birefringence for some hours in the presence of saturated urea. Neutral solutions of the viruses give precipitates with clupein or papain from which the former can be recovered unchanged by resolution at pH 3.3. These precipitates may also be redissolved by addition of salts, the critical salt concentration for resolution depending on the nature of the virus (Bawden & Pirie, 1937c).

The plant viruses are good antigens, and the behaviour of their rabbit antisera in precipitin and cross-absorption tests has been used by Bawden & Pirie as a means of distinguishing between virus strains.

XI. SUMMARY

1. By fractional precipitation of the crude sap from virus-infected plants, or by ultracentrifugation, protein substances are obtained which are capable of transmitting the disease. These proteins may be purified by various methods, notably by incubation with trypsin which selectively digests the impurities. They have high phosphorus contents and behave as nucleoproteins.

2. Examination in the ultracentrifuge reveals that these proteins have larger molecules than any hitherto discovered. In carefully prepared specimens the sedimentation boundaries are those of perfectly homogeneous substances. Sedimentation constants varying from 115×10^{-13} to 240×10^{-13} have been obtained for different viruses.

3. The viruses are stable inside the range pH 2.0-9.0, although showing often an insolubility zone. They are electrochemically homogeneous inside the stability range with isoelectric points somewhere between pH 3 and 5.

4. One virus, that causing Bushy stunt disease, has a spherical molecule and gives regular crystals. The others are anisotropic and give ill-defined needles. Purified solutions of the anisotropic viruses show marked birefringence of flow, and this phenomenon in conjunction with X-ray analysis has been used to obtain estimates of the molecular parameters. In the case of the tobacco mosaic virus the thickness of the molecule is 15 m μ . and the length probably 40 times this value. Bushy stunt virus has a diameter of 27 m μ . and a molecular weight of 8,800,000.

5. The crystallinity of the viruses cannot be regarded as a proof of their purity. X-ray measurements on the crystals show that these have the intramolecular pattern characteristic of proteins. Measurements on semi-dry films of virus show that in these the molecular rods are closely packed together with very little space for water or any foreign substance between them.

6. Some of the viruses may exist in the crude sap in smaller molecular forms than in the purified state. Agents are described which are capable of destroying the infectivity of the viruses without affecting their molecular size or ability to crystallize.

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ADDENDUM

The vexed question of the role of nucleic acid in the plant viruses is settled by Stanley's withdrawal of previous criticisms, and his acceptance of the view put forward by Bawden & Pirie that the nucleic acid portion of the virus is essential to its activity (Stanley, 1938*a*). Loring, Lauffer & Stanley (1938) find that repeated centrifugation of tobacco mosaic virus has little or no effect on the activity, double refraction of flow and filterability of the virus. They can find no evidence for the view put forward by Bawden & Pirie that in the course of the isolation of the virus unavoidable aggregation takes place. On the other hand the American workers agree that subsequent treatment of unaggregated virus with salts leads to aggregation. Bawden & Pirie (1938*e*) do not entirely agree with these conclusions and Smith & MacClement (1938) dispute the interpretation of the filtration data.

Stanley (1938*b*) reports the isolation of aucuba mosaic virus from infected tomato roots grown in isolated culture. It is thus evident that the multiplication of virus in the plant cell is independent of the respiratory mechanism of the normal plant. The aucuba virus prepared in this way appears to be essentially the same as the virus isolated from diseased tobacco plants grown in the usual greenhouse conditions. The work of Bawden & Pirie on latent mosaic virus has been confirmed and extended by Loring (1938*b*). The virus grown on the tobacco plant has a higher specific activity when prepared by ultracentrifugation than by salt precipitation. The two preparations have the same serological properties and both give liquid crystalline solutions. The less active salt preparations however show much more diffuse sedimentation boundaries and the suspensions are more opalescent. There is evidence that the loss of activity is associated with loss of carbohydrate and phosphorus from the molecule. Loring confirms the relative instability of the virus, his solutions showing a drop in activity after 1 day at room temperature and pH 7 and being inactivated immediately on drying from the frozen state. The isoelectric point in 0.01 *M* pathalate-phosphate-borate buffer varies from 3.7 to 5.0 according to the previous treatment of the virus. The stability range is pH 4 to 10, the virus being apparently more stable than the tobacco mosaic virus in the range pH 8.5–10. Ross & Stanley (1938) claim that partial inactivation of tobacco mosaic virus by formaldehyde is a reversible process.

Further attempts have been made to obtain precise information with regard to the molecular dimensions of the plant viruses. Neurath & Saum (1938) have made measurements of the diffusion constant of tobacco mosaic virus using diffusion periods up to 12 days. The diffusion curves obtained by Lamm's refraction method are asymmetrical and their interpretation doubtful owing to the effects of orientation. A value of 3×10^{-8} sq.

cm./sec. is obtained for the diffusion constant and from this and the sedimentation constant a value of 59 millions is derived for the molecular weight. Using Perrin's equation (1.0) the axial ratio is determined as 1 : 55. Lauffer (1938*b*) reports a study of the viscosity of tobacco mosaic virus solutions over the pH range 2.1-10.6. There is a minimum at the isoelectric point and a fall in more acid and alkaline regions. The view is put forward that the aggregation of tobacco mosaic virus in the instability zone is a complex phenomenon of side-to-side aggregations in the region of the isoelectric point and end-to-end aggregations outside this. This is in line with the absence of any corresponding minimum of double refraction of flow at the isoelectric point since this property which is dependent on the asymmetry of the molecules will be but little affected by side-to-side aggregation. The author suggests that in these preparations of tobacco mosaic virus which show two boundaries one ($S_{120} = 174$) is due to single molecules and the other ($S_{120} = 202$) to double molecules formed by end-to-end associations. Loring (1938*b*) suggests a similar explanation for the two boundaries ($S_{120} = 113$ and 131) often found in preparations of latent mosaic virus. This author on the basis of sedimentation and viscosity measurements finds that the axial ratio of the latent mosaic virus is 1 : 44 and the molecular weight 26 millions. Bernal, Fankuchen & Riley (1938) have obtained X-ray powder photographs of Bushy stunt crystals in their mother liquor. These show that the crystal cell is a body-centred cubic lattice of side 394 Å. This corresponds to a particle of radius 17 mμ. The density of the wet crystals is 1.286 and assuming two particles per cell the molecular weight is 24 millions. The crystals shrink visibly by 80% on drying and this shrinkage is confirmed by X-ray measurements on the dry crystals. Using McFarlane & Kekwick's value of 1.35 for the dry density, the molecular weight in the dry state is 12,800,000 or approximately 50% more than the value arrived at by centrifugal methods. The value of 1.35 is the density of Bushy stunt virus dried over phosphorus pentoxide. It is possible that this value is too high for crystals dried in air.

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THE FUNCTIONS OF INSECT BLOOD

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(Received 22 September 1938)

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I. INTRODUCTION

IN insects the general body cavity is a blood sinus. In this cavity, or haemocoel, the blood circulates, making contact with all the organs of the body. The blood is the only tissue fluid. Most of the respiration in insects is carried out by the tracheal system, which conveys oxygen directly to the tissues, and which (perhaps to a less extent) removes carbon dioxide from them. Any respiratory function of the blood is clearly of a subordinate character, and the blood cannot be divided into "venous" and "arterial" as it can in all those animals in which it is the medium for conveying the respiratory gases. Insect blood serves to transport food and other (e.g. excretory) substances about the body, and it has numerous special functions which are carried out in ways peculiar to the group.

The circulatory system of insects contains few discrete blood vessels. Usually there is a single dorsal vessel which runs along the length of the abdomen and

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thorax, and leads into the head. The hind part of this vessel forms the heart, which possesses a system of ostia and valves. The heart is contractile, drawing in blood from the abdomen and driving it forward through the non-contractile anterior portion of the dorsal vessel (the aorta) into the head where the fluid is liberated into the haemocoel near the brain. There may be accessory pulsating organs which aid circulation in the veins of the wings or through the antennae. For the rest, the haemocoel is merely divided up by various membranous diaphragms which direct the flow backwards through the thorax, through the cavities of the legs, and back to the abdomen and the heart. Considering its simplicity, the circulation is often surprisingly efficient (Freudenstein, 1928; Brocher, 1931).

The amount of blood present in the body of an insect may be as low as 15 % of the total weight, or as high as 70 %. There are some species which normally have a large blood volume, and others which normally have a small volume, but the quantity fluctuates enormously even in the same individual. This whole question is discussed in detail below (see p. 250).

The blood varies, not only in volume, but also in its physical and chemical properties, even in the same insect in the various developmental and nutritional stages. As in most other animals the blood consists of corpuscles and plasma, both of whose functions will be discussed below. While the morphology of the heart and associated structures has received some attention, and many analyses of the blood plasma have been recorded, few workers have attempted to investigate the functions of the blood from the physiological aspect. Yet the blood, bathing the organs and tissues of the insect, plays an important part in every one of its activities. Most of the information given in this article has been derived from accounts of observations of these various other activities, where the authors have recorded facts bearing on my subject.

II. THE HAEMATOCYTES

(1) GENERAL DESCRIPTION

Insects never possess corpuscles which are respiratory in function; all the haematocytes seem to be homologous with vertebrate leucocytes. Both in newly shed blood and in preparations the corpuscles give the appearance of many different forms, but further examination shows that one particular type of cell may vary enormously in appearance, and it is possible that there is only one type of corpuscle with a different appearance at different stages in its development.

Embryologically the haematocytes arise from the median mesoderm at a comparatively early stage, before the embryo has closed dorsally and before the heart has been fully formed (Nelson, 1915; Eastham, 1930; H. Mellanby, 1936). They appear to have been found arising only in this one site, and during embryonic life the corpuscles are not apparently differentiated. In later life they multiply by dividing mitotically (Wigglesworth, 1933*b*), though certain workers, having seen few mitoses (Haber, 1926), consider that the rapidity with which the number of circulating cells sometimes increases means that they must arise in some special tissue and not in the blood itself (Taylor, 1935). Wigglesworth (1933*b*) has shown that the

cell divisions are much commoner at certain times than at others, and that usually many of the corpuscles are found attached to the basement membrane of the hypodermis. When required they are able to detach themselves into the blood in large numbers. The assumptions of Haber and of Taylor therefore appear unnecessary, for these workers did not realise that the majority of the haematocytes might merely be adhering to the basement membrane and thus were not always shed with the blood for examination.

It is actually unusual for all the haematocytes to circulate with the blood. Not only may they remain temporarily attached to the basement membrane, but permanent or semi-permanent aggregations known as "phagocytic organs" occur. Lange (1932) has shown that in larvae of the Chironomidae some forms have no free blood cells, for all the corpuscles are found in these aggregations. Other larvae from the same group have some circulating haematocytes, and yet others have practically all of them free. In all these chironomid larvae the haematocytes become free during the pupal period.

There have been many cytological descriptions of the haematocytes (Hollande, 1909; Chorine, 1931; Wigglesworth, 1933*b*), the results of which do not entirely agree with one another, but it seems safe to say that, apart from immature cells and those in process of division, only two distinct types can be recognized, and these occur in practically all insects. The first group (usually containing the majority) comprises basophil *phagocytes*, and the second the *oenocytoids* which are rounded, eosinophil, non-phagocytic cells, whose function is unknown. It is, however, not impossible that the phagocytes and oenocytoids are different stages in the life of the same cell, a view which seems to be supported first by the absence of any circulating oenocytoids in the blood of those chironomid larvae possessing only phagocytic organs, and secondly by embryological evidence. Lartschenko (1933) further considers that some of the cells identified by other workers as immature phagocytes are mesenchyme cells without phagocytic properties, but this view has not received general acceptance. Finally, old degenerating cells and phagocytes charged with ingested substances may easily be mistaken for new types of cells.

(2) FUNCTIONS

(a) *In normal development*

Pérez (1910) in his classical work on metamorphosis in the blow-fly, *Calliphora erythrocephala*, showed the importance of the phagocytic haematocytes during the pupal period. In this insect practically the whole of the larval tissue is broken down and reconstructed, and Pérez found that the phagocytes are the principal agents in disrupting the larval muscles, invading, digesting and then setting free their constituents to be reconstituted into imaginal tissue. In *Calliphora* the phagocytes appear to initiate the breakdown of the larval muscles, for they attack them before any histolysis has begun. It is possible, however, that the phagocytes are actually attracted by some of the first chemical products of breakdown, given out before any visible changes have occurred. This view is supported by the investigations of

Evans (1936*b*) on metamorphosis in the allied *Lucilia sericata*. In the pupa of this insect the phagocytes do not attack the larval tissues until after visible histolysis has begun. But whether or not the phagocytic blood cells initiate the breakdown processes in different pupae, in most endopterygote insects they play an important part at some stage in metamorphosis. A few cases have, however, been described in which there appears to be little phagocytosis at any stage in the pupa (i.e. the bee; see Snodgrass, 1925).

Considerable histolysis occurs, not only in the endopterygote pupa, but also at each moult in both endopterygote and exopterygote insects. Wigglesworth (1933*b*) has shown that the phagocytes in the bug *Rhodnius* become most numerous at each moult, when they ingest dead cells and tissues. It seems probable that the main normal functions of the phagocytic haematocytes is the removal of dead tissues, a view which is supported by the fact that in the adult *Rhodnius*, where there is no more reconstruction to be made, they are much less numerous than in the earlier instars.

Lazarenko (1925, 1928) considers that the phagocytes in the blood are responsible for producing membranes and connective tissue. This view is not held by Lartschenko (1933) who considers that it is not the phagocytes, but "mesenchyme" cells (also circulating in the blood) which are responsible. However, as Thorpe (1936) has pointed out, Lartschenko's distinction between phagocytic and mesenchyme cells is not completely satisfactory, and he considers that it is probably the phagocytes which produce the membranes. Wigglesworth (1933*b*) found that many of the phagocytes are normally adherent to the basement membrane, suggesting that they might have produced it, but his experiments on the healing of wounds (1937) show that in *Rhodnius* under these circumstances the basement membrane is produced by the cells of the hypodermis. Friedrich (1930) found that in limb regeneration in the stick insect, *Carausius*, practically all tissues, including membranes, are produced by the hypodermis. This shows, I consider, that the cells of the hypodermal layer may retain considerable plasticity, and, as in the regeneration in *Carausius* the tissues are produced in a manner markedly different from that found in normal development, so also wound healing in *Rhodnius* may show abnormalities in membrane formation. But whichever of the views expressed above is correct, even if the blood cells do not produce the basement membrane it seems certain that at least some of the internal membranes in insects are produced by cells circulating in the blood, probably by the phagocytes, but perhaps by undifferentiated mesenchyme cells of similar embryonic origin.

(*b*) Protective functions

It is well known that if substances such as Indian ink or carmine are injected into an insect's body, they are rapidly ingested by the phagocytic blood cells. This is believed to be the reaction of these cells to invasion of the body by any foreign object, though the attraction of the phagocytes to any invading substance appears to be chemotactic, and if invasion takes place without the liberation of attractive substances, then the phagocytes do not attack the invader.

Insects suffer from bacterial diseases. Many of the bacteria which cause disease in man, however, are not pathogenic to insects, and these organisms, if injected into the haemocoel, are quickly ingested by the phagocytes and then digested (Metelnikov, 1908; Chorine, 1931; Cameron, 1934). Some bacteria such as *Corynebacterium diphtheriae* attack both men and insects, whilst there are others which are pathogenic only to insects. Most bacteria produce substances which stimulate the haematocytes to phagocytosis, and whether or not an insect succumbs to a bacterial disease depends upon whether all the bacteria are consumed before they multiply and destroy the insect. Chorine (1933) has shown that the temperature and humidity of the environment may affect the phagocytic resistance of insects and the course of bacterial disease in their bodies. Hot and dry conditions which are unfavourable to the host allow the bacteria to get the "upper hand"; under more favourable conditions the parasites are all killed. Metelnikov (1908, 1932¹) and his colleagues have extensively studied the question of bacterial diseases and immunity in insects. It appears that it is possible by processes analogous to vaccination in vertebrates to render insects immune to attack from bacteria known to be pathogenic (Zernoff, 1928; Chorine, 1931). In an immune insect the phagocytes attack the bacteria more rapidly and successfully than otherwise. Immunity may be conferred within a few hours of vaccination, and may last only a day, or for several weeks, or the property may even be transmitted by the mother to her offspring. According to Metelnikov and his co-workers, the immunity is in some way regulated by the nervous system. Extirpation of the third thoracic ganglion may prevent the production or persistence of immunity, though removal of the brain does not have this effect.

In diseases caused by Protozoa, the phagocytes behave much as they do against bacteria. Chorine (1930) has shown that pathogenic Protozoa will only develop successfully in their proper insect hosts, where they are only attacked to a limited extent. In other insects, though the parasites may show some slight development, they are quickly overcome by the haematocytes.

It was previously believed that the phagocytes helped to protect insects against parasites belonging to higher groups as well as against Protozoa and bacteria. However, as Thompson (1930) has shown, many insect parasites are ignored by the blood cells, and these observations have given rise to a belief that healthy parasites are never attacked, presumably because they do not cause attractive substances to be produced. There is no doubt that dead or unhealthy parasites are attacked. While some parasites are left untouched in the haemocoel, many others are surrounded by phagocytes (or, according to Lartschenko (1933), by mesenchyme cells), and eventually they are covered with a capsule produced by the blood cells. The effect of the capsule is different in the case of different parasites. Sometimes the capsule provides a successful protection to the host. Lartschenko has described insects which exhibit "non-susceptibility" (*Unempfänglichkeit*) towards certain parasites, for they cover them with complete cysts which may be extruded at the next moult (Lazarenko, 1928), or which may remain within the body without harming the host. Then Strickland (1930) finds that the tachinid, *Gonia*, which has a complicated life

¹ *Biological Reviews*.

history inside certain noctuid caterpillars, may be attacked by the haematocytes at a critical stage. The eggs of the parasite are swallowed by the caterpillar, and hatch inside its stomach. The tiny larvae bore through the gut wall into the haemocoel and make their way to the brain, being unable to feed until they reach it. The successful larvae are not molested either on their way to the brain, or in a later stage when they lie in the haemocoel, but many young individuals are attacked by the haematocytes and encapsulated so that they cannot reach the brain. They die from starvation inside the cysts. It is probable that many other unsuccessful parasites are overcome by the haematocytes. When breeding insects these forms are probably often overlooked, only the successful parasites which escape attack and emerge being discovered.

There are other cases where the capsule produced by the host is either harmless or of definite use to the parasite. Trematodes are usually encapsulated without any adverse effect to them (Hollande, 1920). As Thorpe (1936) has shown, there may be a very complicated relation between the host and the parasite, leading to conditions in which the host produces a cyst essential to the parasite for its well-being. For instance, when the coccid, *Saissetia*, is attacked by the chalcid, *Encyrtus*, though in the early stages the parasite lies untouched in the haemocoel, at a later stage it becomes invested by a capsule constructed by the host in a manner which allows the parasite's tracheal system to make contact eventually with the atmosphere. It would appear that the phagocytic or capsule-forming reactions of the haematocytes must in the first instance have been protective, and successful parasites are those which have overcome these reactions and even used the capsules for their own purposes. When a parasite is not attacked it is because it is adapted to a particular host. In another host it would be overcome; the action of the haematocytes helps to account for the specificity of parasites. It may sometimes be that a parasite in an unsuitable host is first adversely affected by the general conditions so that it becomes unhealthy and then attracts the haematocytes, but in such cases they are still useful in removing or isolating the moribund invader.

The phagocytes have been considered to be of importance in the healing of wounds, but the work of Friedrich (1930) and Wigglesworth (1937) indicates that the hypodermis is the source of most of the regeneration. The phagocytes may collect round the wound and help to clean up the dead tissues.

Not all foreign bodies in the blood are attacked by the haematocytes. After starvation and desiccation the gut of the bed-bug may become torn, and on feeding, the blood corpuscles of the mammalian prey may escape into the haemocoel and circulate in the insect's blood for weeks unchanged (Wigglesworth, 1931*c*). Presumably they do not emit substances which attract the phagocytes. Implants of living tissues from the same or allied species can live and develop without molestation by phagocytes (Ephrussi & Beadle, 1935*b*; Wigglesworth, 1936), though implants from unrelated species may be the centre of considerable haematocytic activity (Ries, 1932).

It is possible that the phagocytes help in some way in the insect's resistance to inorganic poisons. Pilat (1935) found that when locusts are poisoned by eating sub-

lethal doses of sodium arsenite or silico-fluoride, the haematocytes show considerable mitotic activity. It is difficult to understand how such protection could act. The phenomenon may merely be a reaction of the blood cells to substances liberated by the poisoned gut cells.

III. THE BLOOD PLASMA

(1) GENERAL DESCRIPTION

The haematocytes form only a very small proportion of the blood ($\frac{1}{10}$ th or considerably less of the volume, Muttkowski, 1923), the remainder being the plasma. The plasma is a more or less viscous liquid, which may be coloured or colourless. Its properties have been determined by a number of workers for different insects, and the most noticeable feature is the variability in chemical constitution even in the same insect at different times. Mammalian blood remains remarkably constant in its constituents and reaction. This is necessary because the transport of oxygen and carbon dioxide is a very important function, and any change in the properties of mammalian blood would alter its capacity for these gases. In the insect, where most respiration is carried out by the tracheal system, it is less disastrous when the composition of the blood alters considerably.

The plasma normally seems to contain some proportion of all the elements considered necessary to life, as well as numerous organic substances. It is not proposed to give here details of analyses, which in our present state of knowledge appear somewhat as a series of disconnected facts with little relationship to the life of the insects. Full accounts will be found in the papers by Bishop *et al.* (1923, 1925), Heller & Moklowska (1930), Duval *et al.* (1928), and Geyer (1913). Determinations of vapour pressure are described by Fox & Baldes (1935).

Embryologically the plasma first appears as tissue fluid above the mesoderm and below the yolk, in the space into which the blood cells proliferate. It is produced as the result of the metabolism of the fatty yolk. The fluid is kept replenished in later life from the food (and liquid) in the alimentary canal, and also by the water of metabolism from the breakdown of fat and other reserves.

(2) FUNCTIONS

(a) *Water storage*

Insects are the only really small animals which can live under dry conditions. This is partly because they are so constructed as to be able to conserve water and at the same time carry out their normal metabolic processes (Buxton, 1930, 1932, 1933; Gunn, 1935; K. Mellanby, 1932*a*, 1935*b*). As in other animals, the insect's protoplasm contains a high proportion of water, and it is necessary for active life that this high proportion should be maintained. Now the blood plasma of insects consists mainly of water, and this water may serve as a reserve which can be drawn upon when needed. Buxton (1933) has divided insects into two physiological classes which he describes as "Spenders" and "Savers". The spenders normally live in moist environments, consume food with a high proportion of water, and do not

conserve water to any great extent. Such insects usually feed almost continuously, or at frequent intervals, and their blood remains fairly constant in volume and composition. The second group, the savers, consists of those insects which can withstand desiccation. They may take dry foods, such as flour and stored products, but many of them suck mammalian blood. Now though mammalian blood contains much water, it is usually dealt with so that it becomes, as far as the insect is concerned, a "dry" food. Blood suckers tend to take enormous meals at infrequent intervals; immediately after feeding practically all the water is extracted from the meal and excreted (otherwise its weight would interfere with the mobility of the insect) so that a comparatively dry residue remains. If a blood-sucking insect is examined shortly after feeding, it will be found to have a great deal of circulating fluid in its haemocoel. After a period of desiccation and starvation it will be found that of the insect's own blood only a fraction remains, and this is much more concentrated (Wigglesworth, 1931c; Winogradskaja, 1936). The conditions in the tissues themselves do not seem affected by the dryness, for the blood has given up its water to maintain them (K. Mellanby, 1937). This is further shown by the way in which the rate of metabolism at any particular temperature in a resting insect is seldom affected by considerable alterations in its total water content, caused by exposure to different conditions of atmospheric humidity (K. Mellanby, 1932a, b, 1934, 1935a, b, 1936a, b) though the behaviour of active forms may be affected (Gunn, 1934, 1935). When a "saver", particularly if it is a blood sucker, feeds once more after a period of desiccation, it uses some of the surplus water in its food to restore the volume of its own circulating fluid (K. Mellanby, 1935a).

Incidentally, during the pupal stage of all endopterygote insects the animal, because it does not feed, becomes a "saver". The pupa has to depend on water present in its body at the beginning of that stage, and on water produced by metabolism of its other reserves. Many pupae are found in damp situations where desiccation is unlikely, but the water in the blood may serve as an important reserve (Heller, 1932).

The fact that the blood is not primarily respiratory, and may therefore have its composition greatly altered, allows insects and tracheate arthropods to withstand such high degrees of desiccation.

(b) *Transportation*

The principal function usually attributed to insect blood is that of transporting the products of digestion in the body. This is certainly most important, and essential to prolonged activity, though it is possible for a desiccated individual containing so little blood that there is no proper circulation to survive for a long time in a dormant condition. If such an insect is made to exercise itself, it soon dies from exhaustion unless its reserves are replenished. The insect dies, not because all those reserves which would last the dormant individual for a long period are exhausted, but because the vital tissues are affected, and the lack of circulation prevents their rapid restoration. In dormancy, diffusion alone is sufficient to transport the small quantities of nutriment required (K. Mellanby, 1938a).

The method of circulation in insects, in which the general direction of flow is such that the blood is drawn by the heart direct from the abdomen to be discharged by the aorta in the head, is perhaps analogous to the mechanisms found in vertebrates for ensuring a supply of well-oxygenated blood to the brain. It causes the insect's brain to receive the blood when it is purest and most highly charged with nutritive substances, because in the abdomen the excretory products previously discharged into the blood are removed by the malpighian tubes and the nutritive content is renewed from the gut or from reserves.

✓ Little information exists regarding the changes in composition of the blood in connexion with nutrition. The early stages of fat digestion are known to give rise to many minute globules of fat ("lipomicrons") in the circulation (Haber, 1926), and digestion of other substances must cause characteristic changes. Alterations in composition of the blood occur during starvation, for instance the osmotic pressure may be reduced (Wigglesworth, 1938) provided there is no desiccation when the opposite effect is obtained.

✓ Not only nutritive and excretory substances are carried by the blood. Experiments of Wigglesworth (1934*a*) and Fraenkel (1935) have shown that moulting and metamorphosis are controlled by hormones, probably secreted by the corpus allatum. These hormones are discharged into the blood, and carried by it to the tissues. At some period the blood must contain considerable amount of hormone, for if the fluid is drawn off at the critical stage from one insect and injected into another, it is able to produce changes in the second individual, sometimes even inducing an almost normal moult at a quite unusual stage of development. If in the original animal the substance were being used up as rapidly as it was produced, injection of blood would not have this effect. There is evidence that under cold conditions, which inhibit metamorphosis, the hormone may remain unchanged in the blood for long periods, though high temperatures may cause its destruction without necessarily injuring the organ which produces the substance (Mellanby, 1938*b*).

✓ Substances whose chemical nature has not been determined, but which are concerned in the control of eye colour and other heritable characters whose transmission may be studied genetically, are carried by the blood in the fruit fly *Drosophila* (Ephrussi & Beadle, 1935*a*).

(c) Food storage

✓ The fat-body is the principal food store in insects, but at times the blood may contain considerable quantities of reserve materials. Heller (1932) found that during the pupal period in the moth *Deilephila* 62 % of the solid matter utilized was taken from the insect's blood.

✓ The blood of the bee larva contains little glucose (Bishop *et al.* 1925), but Beutler (1936) has shown that adult bees may have up to 4 % of this substance in their blood. This difference may be connected with the difference in mode of life of the larva and the adult. The bee larva always remains almost quiescent, but adult insects, when they fly actively, may increase their rate of metabolism by over a thousand times (Kalmus, 1929). This enormous increase in metabolic rate requires

time that they extrude blood these insects usually "feign death". The methods of extrusion of blood are various, some forms merely rupturing their cuticle at a weak spot, while others have special pores and preformed mechanisms for the purpose. Hollande considers that most of the preformed openings evolved first of all as glandular orifices, and later took on their peculiar defensive function in reflex bleeding. In many cases when the danger passes the extruded blood is not lost; the pressure in the circulatory system is normally less than atmospheric, so the fluid is drawn back into the haemocoel when the muscles which caused the bleeding relax.

Certain moths defend themselves by producing a nauseous froth. This consists largely of blood and air, though the noxious properties may be due to a glandular secretion (Carpenter & Eltringham, 1938).

(ii) *Immunity.*

As mentioned above (p. 247), immunity in insects usually depends on the efforts of the haematocytes. It is difficult to be sure, however, that the blood plasma does not take some part in this reaction, perhaps by virtue of the presence of substances which activate the phagocytes or cause them to multiply. Certainly in some cases if the corpuscles alone (without any plasma) are transferred from an immune to an ordinary insect, the immunity is also transferred (Zernoff, 1928), but in most the plasma is believed to play an important part. In mammalian blood, the constitution of the plasma may affect the electrical charge on invading organisms, for instance the negative charge on bacteria may be reduced; this is a necessary preliminary to phagocytic ingestion (Brown & Broom, 1935). Changes in the electrical charges due to plasma-constitution would explain many experiments on immunity in insects, for instance those in which the speed of phagocytosis is greatest in immune forms, and this would indicate that the plasma is as important as the haematocytes.

Chorine (1931) has shown that humoral immunity exists, and can be produced in non-immune insects, but up to the present it would appear that this is subordinate to the phagocytic reactions.

(f) *Mechanical functions*

By contraction of one part of an insect's body, pressure can be transferred to another. The blood is the means by which this pressure is transmitted. Inflation of the requisite part of the body helps in emergence from the egg, in moulting and in escaping from the pupa. Many references on these points are given by Wigglesworth (1934*b*). The wings in the newly emerged adult are commonly dilated by blood pressure. In the Muscidae the ptilinum, an eversible sac in the front of the head which helps the adult to escape from the puparium and to progress through the soil, is actuated by blood pressure (Laing, 1935; Fraenkel, 1936).

In a previous section (p. 249) it was shown that insect blood serves as a reserve of water, which can be utilized during periods of desiccation. Sometimes this use prevents some later part of the development from being properly carried out. When tsetse pupae are kept under dry conditions they may complete their development,

drawing on the reserves of water in their blood, but so much of the fluid may be used up that there is insufficient volume left to enable the insects to expand sufficiently to escape from the puparium. If the top of the puparium is artificially loosened, the fly is able to escape (K. Mellanby, 1936*b*). Under dry conditions many insect eggs complete their development but fail to hatch. The failure must often be due to there being too small a volume of blood. There appears to be a rather delicate partition between the satisfactory use of the water in the blood as a reserve in dry conditions, and its use mechanically at a later stage. This is just another instance of the way in which the well-being of the insect is dependent on the climatic conditions of environment.

When larvae of the blow-fly, *Lucilia*, are kept under dry conditions, the volume of the blood is considerably reduced and the larvae shrink noticeably. If some of these larvae are put into moist earth, they drink water, and the water diffuses through the gut wall and replenishes the blood, restoring the larva to its original size. If the desiccated larvae pupate, they produce small pupae, while those which have drunk produce larger ones. During the pupal period the whole construction of the tissues is remodelled, and the size of the resulting adult depends on the size of the limiting puparium. The desiccated larvae give rise to smaller flies (with smaller wings and smaller sclerites) than those which have drunk, though both have the same dry weight (K. Mellanby, 1938*b*). It should be noted that the larger fly is the "normal" one, for the prepupae generally occur in moist soil, but it is the blood that is responsible for maintaining its size. It may be remarked that Holdaway & Smith (1932) have found that the size of the pupa formed by a number of muscids, including *Lucilia*, has a definite relationship to the sex ratio of certain hymenopterous parasites (*Alysia*) bred from the pupae. Thus the blood, by its control of pupal size, may have an indirect effect on the parasite population and so on the future of the race. Cretschmar (1928) states that the different wing sizes found in the various forms of females of the moth, *Orgyia*, are due to the presence of different quantities of blood. The factors which control the blood volume (and so the wing size) are heritable. It seems probable that the external size of many other insects is in some way controlled by the volume of the blood.

IV. CLOTTING OF THE BLOOD

As clotting may be a function of both the haematocytes and the plasma, it is here considered in a separate section. In mammals the blood is normally under a positive pressure, and unless it clotted even a small wound would be fatal. In insects, the position is different. Though the blood may, by muscular effort, be raised to a considerable positive pressure, under most circumstances it has a pressure less than atmospheric, so that little is lost at a wound (Brocher, 1931; Hollande, 1911). This, together with the fact that the resistant exoskeleton possessed by most insects makes small wounds uncommon, means that the possession of blood which clots is not necessary to all members of the group.

Many insects actually possess blood which does not clot. Species of this kind

occur in many orders. With them, a small amount of blood oozes out at a wound, where it dries into a resistant scab which may eventually be transformed into cuticulin, the very resistant outer covering of the cuticle (Wigglesworth, 1937).

If blood clots at all, this may happen in two different ways. In the first, the haematocytes tend to aggregate and send out a fine network of pseudopodia. The plasma does not in this case clot, but eventually dries among the network, which is so dense as almost to occlude any wound (Yeager *et al.* 1932). This type of cellular clot is very different from that found in mammals. Calcium is not necessary for its formation. It is possible that those insects whose corpuscles do not take part in the formation of a clot do not actually behave in a completely different way, but that they do aggregate to some small extent, and the difference is merely one of degree.

In the second type of clotting the plasma forms the clot, with more or less aggregation of corpuscles (Yeager & Knight, 1933). This type of clotting appears more closely to approach that found in the vertebrates.

Even with those insects whose blood does clot, the time taken may be so great that the process cannot be of much protective value, and, as stated above, clotting is not really necessary in this group. In fact it might sometimes be a disadvantage. In those forms which protect themselves by "reflex bleeding", if the blood clotted rapidly they would lose the whole volume extruded instead of being able to draw most of it back into the haemocoel.

V. SUMMARY

Insects have a haemocoel, in which the only tissue fluid, the blood, circulates. The blood consists of haematocytes and plasma.

Most of the haematocytes are phagocytic leucocytes. These alter considerably in appearance at different stages of development, assuming many different forms. Respiratory corpuscles are never present.

The phagocytes assist in breaking down obsolescent tissues. They are most active during the pupal period in endopterygote insects, but also exhibit considerable activity at each moult in both endopterygote and exopterygote forms. Haematocytes take some part in producing internal membranes.

The phagocytes serve to protect the insect's body against invasion, particularly by bacteria. Immunity against bacterial diseases can be developed, either naturally or by processes analogous to vaccination, and in immune insects the phagocytes attack the bacteria more rapidly and more successfully than otherwise. Metazoan parasites may be overcome by the haematocytes, which then surround the invader with a capsule. Parasites which are adapted to living inside specific insect hosts suffer no inconvenience from the activities of the blood cells. The capsules produced may even be necessary for the well-being of these parasites.

The plasma is a viscous fluid which may be coloured or colourless. Except for haemoglobin in solution in the plasma of chironomid larvae, no respiratory pigments are found. The fluid consists mainly of water, but the percentage of dry matter, the proportion of many of the various constituents and the reaction of the

liquid shows considerable variations even in the same insect at different stages of development.

The water in the plasma serves as a useful reserve, and allows the insects to withstand considerable desiccation. Under such circumstances the blood becomes more concentrated and more viscous.

Food substances are transported and stored in the blood, which also carries hormones about the body.

Respiration in insects is primarily the function of the tracheal system, but the blood has certain subsidiary functions. The tracheoles do not enter all tissues, and some cells receive their oxygen from solution in the blood, which serves as an intermediary between the tissues and the tracheae. Various structures have been described as being especially adapted to oxygenate the blood, but these have mostly been found to be unimportant. The osmotic pressure of the blood may govern the extent to which air extends into the tracheoles. Muscular activity liberates metabolites which increase the blood's osmotic pressure, and this removes some fluid from the tracheoles and draws the air up among the active tissues, thus increasing their oxygen supply.

The blood sometimes contains poisonous substances; "reflex bleeding" may then protect the insect from attack.

The plasma as well as the haematocytes may be concerned in producing immunity to bacterial infection.

The blood has important mechanical functions. It is the means by which pressure is transferred from one part of the body to another, and thus assists in hatching and moulting. Desiccation, by reducing the blood volume, may interfere with these processes. The volume of blood also serves to maintain the body size, and if the volume is decreased during development an undersized adult may result.

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REVERSIBILITY IN EVOLUTION CONSIDERED FROM THE STANDPOINT OF GENETICS¹

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(Received 17 October 1938)

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I. THE REVERSIBILITY OF GENE MUTATIONS

THE non-teleological character of the process of variation which furnishes the biological material for evolution is nowhere more clearly seen than in a consideration of the direction of mutation. If the direction of evolution were predetermined by the direction of hereditary variations, then the latter would not take place in both of two opposite directions, or, if it did, it would occur more readily in the direction in which evolution had gone. Neither of these conditions is true. For, in the first place, mutation in both of two opposite directions can occur in the case of nearly all genes which have been investigated with reference to this question; and, in the second place, where a difference in mutation frequency in the two directions occurs, the greater frequency is usually in the direction contrary to that which we judge must have been the past evolutionary one.

Probably the first reverse mutations obtained were those of so-called "ever-sporting" genes. Here belongs, for instance, the change noted by Emerson (1911) from the mutable white ("variegated") allele to red in certain strains of corn, although the fact that the red striping was due to somatic mutation was hardly recognized in a clear fashion at that early date. Since then, not a few other genes of inordinately high mutation frequency have been found. In all these cases the frequent change has been that in the reverse direction, i.e. from the mutant gene to or towards normal, not that in the original direction, i.e. from normal to mutant,

¹ Based on a paper read at a discussion on "Reversibility in Evolution" held by the Society for Experimental Biology in London on 21 December 1937. Compare the article by Needham in *Biological Reviews* (1938).

or from mutant to more extreme mutant. This applies not only to the more obviously "ever-sporting genes" but also to some cases of intermediate frequency of mutation, such as that of Blakeslee's dwarf *Portulaca*, in which occasional normal-sized shoots appear on the homozygous recessive dwarf plant. As we shall see later, these special cases fail to illustrate the aforementioned principle that change in the direction of evolution is less frequent than that in the opposite direction, for although it is the "reverse" change which is here more frequent, the direction of this change probably corresponds in these cases to the primordial direction of evolution. But the principle in question does come to light when the more typical kinds of mutations and reverse mutations are reviewed.

In *Drosophila*, the first instance of reverse mutation was the early finding of eosin eye by Morgan (1913), as a partial reverse mutation from white. In 1929, Morgan reported cases of spontaneous reverse mutation of scute and of forked. Timoféeff-Ressovsky (1928) has obtained normal from yellow body colour as a spontaneous reverse mutation, and on various isolated occasions other spontaneous reversals and partial reversals have been found. It is at once evident that, if we exclude "ever-sporting genes", not nearly so many cases of reverse as of "direct" mutation have been found in *Drosophila* or anywhere else. This, however, cannot in itself afford ground for conclusions concerning the relative frequency of mutations in opposite directions. For it is only what is to be expected, even if the mutation rates in both directions are comparable, on account of the conditions under which the mutations are observed. It must be remembered that *Drosophila* as well as any other organism which is bred contains but one or at most a handful of already mutant genes the reverse mutation(s) of which would be detected, as against hundreds of normal genes in which a so-called "direct" mutation would be detected. Hence, with the same mutation rate, we should expect hundreds of times as many "direct" as reverse mutations to have been found. For example, in a white-eyed stock, a reverse mutation, to be observed, must occur precisely in that locus, white, which is already in a mutated condition, whereas a "direct" mutation occurring in any one of hundreds of the loci containing normal genes would be detectable.

Radiation mutations, by reason of their vastly higher frequency, afford a far better opportunity for a study of relative mutation rates in opposite directions than do spontaneous mutations. Moreover, the results from radiation mutations should have some bearing on spontaneous mutations too, inasmuch as experience shows that all types of mutations previously known as a result of their spontaneous origination can be found in radiation experiments also, if a serious search is made for them. In fact, when non-lethal visible mutations not involving detectable rearrangements are considered, they seem to occur in similar relative frequency spontaneously and as a result of irradiation. For example, white arises oftener from normal than does any given white allele of lesser extremeness, both spontaneously and in radiation experiments, mutations at the white locus occur far oftener than those at, say, the carmine locus in both kinds of experiments, etc. In 1928 I reported the obtaining of reverse mutations both of forked bristles and of the scute bristle character in *Drosophila* by means of X-rays. A quantitative study of the

bristles in the reversed scutes, made by Agol in Texas in 1931 (unpublished), failed to show any difference between them and the original normal type. In 1930 Patterson and I reported a quantitative study of reverse mutations, in which it was found that mutations of the forked locus, produced by radiation, occur with approximately the same frequency in the reverse as in the "direct" direction, while, on the other hand, white eye far less frequently underwent reverse mutation, as a result of irradiation, than the normal allele of white underwent mutation to white.

Timoféeff-Ressovsky (1929-33), in a series of elaborate quantitative studies begun at nearly the same time as these, tested a considerable number of loci, both sex-linked and autosomal, and found reverse mutation of mutant genes to be producible by irradiation in the great majority of them. His results were extensive enough to permit of quantitative treatment. The values which he found ranged from the exceptional case of approximately the same value for reverse mutation of the mutant gene as for "direct" mutation of the normal gene to the mutant, as found in the case of forked, to values of about one-fifth to one-tenth as high for the reverse as for the "direct mutation", as found in the case of white. It will be noted, however, that in all these cases the order of magnitude of the mutation frequency in the two directions was either the same, or differed by only one grade (i.e. the frequencies were not found to differ by more than about a factor of ten). White was further notable for the peculiarity that, although the mutation of normal to white occurred relatively often, complete reverse mutation from white to normal was never observed in a single step, although it was possible to obtain the result in two steps, as by getting eosin eye from white and then normal from eosin, and although one apparent spontaneous white-to-normal mutation, which was however untestable, has been reported. With this exception, practically any of the numerous alleles of white studied could change from one to the other by a single mutation.

In considering these facts in relation to the direction of evolution, we should probably count most of the so-called "direct" mutations as being essentially reverse. For most of them, in *Drosophila* at any rate, prove to consist of changes of the gene to a less active or "hypomorphic" state, so far as its effects on the character under observation is concerned. This has been shown by means of special methods (Muller, 1932), involving the production, by radiation, of chromosome fragments containing the genes in question, followed by observations of the phenotypic effects produced by adding or subtracting these fragments, and comparisons of the effects of these "dosage changes" with the effects of the given gene mutations themselves. Such studies show the gene mutations usually to have effects like those of a lessening of the amount of the gene material in question. It seems evident that, in the majority of cases, the original direction of evolution was the opposite of this, unless we take the perverse view, championed by a few mystics, that modern organisms, in general, are degeneration products from some golden age of living matter.

Mutatis mutandis, then, what we call "reverse mutations" would usually be positive changes, giving rise to genes that act more effectively in the production of the given character, and it is changes of this type, rather than changes having effects similar to losses, which must have played a more significant role in the past

evolution. As we should in general expect in the case of changes causing increases in the degree of organization, these "positive" mutations usually (except in the special cases of "ever-sporting genes") occur less often than the seemingly degenerative mutations; yet they do occur with not inordinately lower frequency. Thus they allow of "progressive" evolution, provided selection is acting to sift out the mutations that occur, but they would not be sufficiently abundant, in comparison with the changes of the opposite kind, to force evolution, through the sheer pressure of variation, in their own direction.

As for the question why still further "positive" steps, beyond the normal, are not observed—from normal to a "hypermorphic" type, i.e. to one showing the opposite deviation from that of the ordinary mutant—the answer is that such changes doubtless do occur too, perhaps with a frequency similar to that of the reverse mutations involving origination of normal itself from a hypomorphic mutant, but they could seldom be observed. For, in most cases, the normal gene itself has, at the present stage of its evolution, passed the critical level of activity in the production of its visible effect, beyond which further increase is much more difficult to detect. This is shown by the fact that even a single dose of the normal gene, in its present state, produces sensibly the same phenotypic effect to our eyes as two doses. The probable explanation of this phenomenon, directly related to that of dominance, has been presented by the author elsewhere (1932, 1935 *a*); it would take us too far afield to go into this matter here.

II. THE SIGNIFICANCE OF REVERSIBLE MUTATIONS IN THE THEORY OF THE GENE

The reversibility of mutations has been used as evidence by Morgan (1913), by myself (1921, 1928), by Patterson & Muller (1930) and by Timoféeff-Ressovsky (1929–33) against the view that mutations commonly consist of mere losses of genes.

But the significance of reverse mutations for gene theory goes beyond this. For, as Timoféeff-Ressovsky *et al.* (1935) have pointed out, the fact that mutations are not merely reversible but often revert with a frequency comparable to that of the original mutation raises an interesting point with regard to the mechanism of mutation and the structure of the gene, as well as with regard to evolution. Calculations show that X-rays produce mutations with a frequency such that the ionization of any one of some hundreds of atoms, at least, can result in a mutation of a given kind, and this holds even in the case of reverse mutations. Either then these different initial chemical changes all lead to convergent mutational processes, processes that become canalized in the same direction, so to speak, so as to produce the same final chemical change in the gene, or else many chemically (or at least topographically) different kinds of changes in the gene result in the same sort of change in its mode of expression (through "canalization" of the reactions set up by the gene in the soma). In the latter case the apparent similarity to one another of reverse mutations, as well as that of the original "direct" mutations, would be only a superficial one, not based on a real chemical identity of the genes concerned. The latter situation would, however, presuppose a very peculiar conformation and mode

of action of the gene, in giving rise to reverse mutations. For it would imply that one mutation, involving a change in a given portion of the gene, could readily have its effect exactly reversed by any one of a great number of different subsequent mutations, occurring in various other entirely different locations in the gene.¹ Hence the first-mentioned alternative becomes more plausible, according to which many different initial chemical changes tend to result in the same final change in the gene.

This connotes that the final step, the mutation, is only an indirect result of the original provoking ionization or activation, and that the specific nature of the mutation depends mainly upon certain paths of least resistance, so to speak, to chemical change, that are inherent in the structure of the gene. This inference agrees with my conclusion (1932, 1935 *b*), based upon other grounds, that the original provoking activation may even lie somewhere outside of the gene that is caused to mutate.

This in turn involves another moot question in modern mutation studies, raised by the finding (Muller, Prokofyeva & Raffel, 1935) of minute rearrangements and of their position effects, which so simulate those of "true gene mutations" that at present no criterion remains for distinguishing between cases of these two different classes, once supposed to be so distinct.² According to the more adequate results recently obtained by Belgovsky (*ex lit.*), as in some preliminary unpublished work of my own (confirmed, since original date of writing, by Muller *et al.* 1938), minute rearrangements, like "gene mutations", vary in frequency with the X-ray dosage at a rate significantly less than the square of the latter; in fact, in Belgovsky's work it is evident that the frequency of these rearrangements varies simply as the first power of the dosage, just as that of gene mutations does. It follows that the two or more chromosomal breaks involved in a given minute rearrangement are produced, ultimately, by one and the same ionization. The breakage, then, must be an indirect consequence of the ionization, for the same ion could not have been a component part of the thread at both places at which it became broken. It is to be noted that this conclusion agrees with that of the paragraph above, that gene mutations are only indirect consequences of the provoking chemical change. This provoking change, itself, requires but one electron-hit (activation or ionization). Now the question raised by all this parallelism between the so-called gene mutations and the minute rearrangements is whether or not they represent fundamentally the same phenomenon. That is, are the "gene mutations" really linear rearrangements, similar to the others, but so minute as to be incapable of demonstration as such by present genetic or cytological methods?

¹ As the same authors (1935) have further pointed out, this alternative is least of all reconcilable with that hypothesis of gene structure according to which the gene is compounded of a number of identical parts, e.g. of a group of like molecules, for it is very difficult to see how a given change of one of these parts could so commonly have its effect reversed by some other change of any one of a number of other parts. The other alternative also (that of a canalized mutation process) is difficult to reconcile with such a conception of gene structure. Previous evidence against this conception had been adduced by me (1926, 1928) from my finding that there is no intragenic sorting out of mutated components of the gene, during the cell divisions immediately following the mutation.

² A general review of work on the position effect has been presented by Dobzhansky (1936) in *Biological Reviews*.

If, however, gene mutations should really consist of very minute rearrangements in the linear order of the genetic material, they could hardly consist in mere rearrangements of "whole genes", in the sense in which we have been accustomed to use the word "genes". For this limitation would neither allow the genetic material sufficient potentialities for the evolution of complicated from primitive organisms, nor would it make possible an explanation of how the many different genes had originally become differentiated from one another. If, on the other hand, our conception included the possibility of "intragenic" rearrangements, involving much smaller units than "whole genes" as now thought of, these difficulties for a linear rearrangement view of gene mutation would be greatly diminished. But in that case it would be questionable how we should define the limits of a gene, and whether in fact there was a distinctly segmental arrangement of the smaller elements in question, into groups that it would be appropriate to call individual "genes" at all.

Shortly after my recent raising of these questions (at the Réunion Internationale de Physique-Chimie-Biologie, Congrès de la Découverte, Paris, October 1937), on the basis of studies at the Institute of Genetics, Moscow, on the relation between gene rearrangements and mutations, Goldschmidt independently put forward an essentially similar point of view, although he presented it not so much as a possibility to be inquired into but rather as an inference which already had the weight of evidence in its favour, and he gave to many the impression that he was attempting, through this obliteration of the limits of individual genes, to sweep away the whole gene theory. It does not appear to me, however, that any such possible revision of our concept of the structure of the genetic material would stand opposed to the main theses of the Mendelian, chromosomal, view of heredity, nor of the essentials of the gene theory so far established. For no matter how we may have to redefine our concepts of how much is involved, respectively, in a "unit" of mutation, of interchange, of reproduction, and of functioning of the hereditary material, in the light of evidence concerning its finer subdivisibility—the linear differentiation of the chromosome remains, and the exchangeability of its parts by crossing over, and the fact that these parts reproduce their own structures, independently of one another. Nevertheless, the problem of the degree of continuity or of segmentation of the chromosome remains a real and an important one. And as yet we are not, in my opinion, justified in deciding whether or not what we thought were gene boundaries are different in their nature from the boundaries between smaller constituent parts, lying within what we previously called "a gene". Moreover, the answer may vary according to which criterion for defining a "single gene"—that of mutation, function, crossing over, or reproducibility—is used. This is a matter which still lies open for further investigation.

In the meantime, it must be recognized that the view according to which the gene consists only of a rather arbitrarily delimited region of the chromosome—a collection of more elementary parts not sharply separated from its neighbour—does meet with certain difficulties at present. Important among these is the difficulty that in that case breakage of the chromosome would usually constitute breakage of the "gene", as taken in the old sense, and therefore a destruction or serious

disturbance of the action of this "gene"; following this, it would be a virtual impossibility ever again to reconstitute exactly this same gene by reverse mutation. For true reverse mutation would require a subsequent breakage at exactly the same two places as those at which breakage occurred the first time, and if, as the view in question further implies, there were no preferential places of breakage, or "joints" between the "genes", this coincidence would practically never occur. The fact of reverse mutation, giving an apparently exact restitution, and of its fairly high frequency, thus furnishes some argument for our older view of the discreteness of the individual gene.

III. MUTATIONAL DISINTEGRATION, IN THE ABSENCE OF SELECTION FOR THE GIVEN END-RESULT

We must now leave these more purely genetic questions, for at the present time it is hardly possible to tie them up closely with our main theme. For our main theme the most important point is that mutations are in fact reversible, even though we cannot be quite sure that the reversions usually reconstitute a gene that is chemically identical with the original one. Furthermore, it is important that mutations in the "minus" direction (no matter whether we consider them as "reverse" mutations or not) are usually commoner than in the "positive" direction. Now, even if we assume that the reversions are exact, do these facts of mutation mean that evolution itself is reversible, in a more nearly ultimate sense? Or may it at least be reversible in those cases in which the reversal would involve change in the "minus" direction, i.e. the direction of lesser organization? That is, to take a crude example, would it be possible for the lion and the lamb, if induced to lie down together in mutual harmony for many millennia, finally to dedifferentiate into something like insectivores, and even at last to have offspring by one another?

Perhaps, however, we should not prejudice the case by making it appear absurd. For it is evident that in many cases evolution has in some sense reversed itself, as when terrestrial vertebrates re-enter the sea and become in some ways fish-like. And certainly organs and characteristics tend to degenerate and disappear when long in disuse, like the eyes and the pigmentation of cave animals.

As regards this disappearance of unused parts, we must reckon not merely with selection but with actual mutation pressure, small as it is. Thus, in *Drosophila*, if there is no light and hence no advantage in having pigmented eyes, and if the normal gene at the locus which by mutation gives white eyes had no other present function than aiding in the absorption of light, we should find that, after some 20,000 years, the great majority of individuals of a population of *Drosophila* that had been left in a cave now had white eyes, as a result of mere mutation pressure. For the change red to white occurs once in any given gene at this locus in an average of some 10,000 years, and the change in this direction occurs much oftener than that from white to red. (In making this calculation, we need take no account at all of the accidental spread of some mutant genes and the elimination of others through the differentially high or low multiplication rate of different individuals, independently of whether or not they happened to carry the mutant in question.) In attaining this

white eye, the flies would resemble in this respect the hypothetical pre-insect ancestors in which pigmentation had not yet evolved. They would probably contain at the same time many other eye-colour mutations that would have been disorganizing to the process of pigment formation even if the fundamentally necessary "white locus" had not been affected. Similarly, their eyes would be malformed, reduced in size, or even absent. In a sense, then, this would be a reversal of evolution, although a very chaotic one.

In fact, however, we made an incorrect assumption in postulating that the normal gene at the so-called white locus had no other function than that of producing a pigment for use in vision. The white-eyed flies are, for example, less viable as larvae, before they have eyes, and less fertile as adults (although at high temperatures the "white" larvae have a higher survival than the normal). This illustrates the fact that any gene which has been established for a long time tends to have acquired secondary functions, by a kind of mutational process of incorporation into the whole ontogenetic and physiological system (to be discussed again on pp. 271-3), so that, as I remarked in raising this point in 1918, "*characters and factors which, when new, were merely an asset finally became necessary*".¹ Thus things which have been recently acquired in evolution can relatively readily be lost again, even by mere mutation pressure, when selection for their original effects is withdrawn, but the longer they remain established, the more difficult becomes their loss. This condition holds not only in reverse but also in positive evolution, and accounts for the tendency towards apparently unnecessary recapitulation of phylogeny in ontogeny.

The circumstance above mentioned would tend to hinder the establishment of the white mutation under cave conditions except perhaps after a very much longer time than that above calculated. During this long time other mutations would have an opportunity to become established, that in part took over the functions other than visual of the reaction system found in normal as compared with white-eyed flies, and as they did so the mutation pressure of the white locus would tend to cause it to lose its corresponding activity, except in so far as its different functions were inseparably tied together. This illustrates the still longer-range antithetical principle, that a once necessary structure or process may finally become unnecessary again. For the genetic fractionation of functions above mentioned is particulate, and its particles are ever shifting, even where there is no outwardly apparent change.

It is to be expected that, long before the pigment reaction of the white locus itself had been completely lost, other mutations would have become established in the population, whether by mutation pressure and accidental spread, or by selection with respect to other characters than vision. Among these various mutations which became established, many would necessarily chance to affect pigmentation, and of those that did far more would tend to disorganize the process of pigment-formation,

¹ This taking on of additional functions by genes previously present, caused both by their own mutation and by that of other genes that come to interact with them, leads to a given function, conversely, having its genetic basis become more and more finely divided, as time goes on, among different genes. An example in point is the present state of dispersal of the function of sex-determination, among genes scattered throughout the X-chromosome, following an earlier stage (just after the establishment of sex-determination through heterozygosis) in which there must have been but one effective gene difference-acting to differentiate the male from the female.

and to reduce or eliminate the pigment, than to maintain or increase it. The reason for this is the same as the reason why more mutations are detrimental to life than favourable, and probably the same as why the mutation from red to or towards white in the white locus itself occurs oftener than from white towards red. This reason lies in the fact that organization requires a more special configuration of material and of motion than disorganization, and it is fundamentally the same reason too which, in physics, gives us the increasing disorganization of motion; i.e. the increase of entropy, enunciated in the second law of thermodynamics. In consequence, when selection for any given end-result is relaxed, dedifferentiation with respect to that character or organ follows in its shadow, and this may often entail a kind of reversal of evolution.

IV. THE PARTIALLY FORTUITOUS NATURE OF THE GENETIC BASIS ARRIVED AT THROUGH SELECTION FOR A GIVEN PHENOTYPE

Besides the above, there must often be active selectional processes at work to give a reversal of evolution so far as the phenotype is concerned, but similar restrictions apply here as in the case of relaxation of selection, which tend to prevent the final product of the reverse evolution from being more than superficially similar to the original type. Selection works for certain end-results, being indifferent to the means by which these are attained so long as these means do not conflict with other end-results for which selection is simultaneously working. And in the intricate economy of living organization with its thousands of interacting genes, the (beginnings of the) paths whereby a given end-result may be attained are many and various indeed. Wherefore we find that mutations, or combinations of mutations, in numerous different loci produce what to us appear similar effects, and what indeed, so far as the present needs of the organism are concerned, are actually equivalent effects. If this is true even in the case of a comparatively restricted process, like the formation of the brown pigmentation in *Drosophila* eyes, which can be abolished either by the mutations vermilion or cinnabar or scarlet or cardinal, and probably by combinations of various other mutations, how much truer must it be of a larger and more inclusive process, such as pigment formation in general, or of some actual organ or whole physiological system.

The same consideration applies even in the case of "positive" reversals from a newly arisen hypomorphic mutant condition, as when, starting with vermilion-eyed mutants, we try to obtain the normal red again from them. We may do this by getting precise reverse mutation, of hypermorphic nature, in the vermilion locus itself, but it is not unlikely that our new red will owe its colour change, instead, to a so-called "vermilion suppressor", or combination of partial "suppressors", since there are various other loci than that of vermilion itself whose mutation will result in normal red pigment, despite the presence of the primary vermilion gene. Similarly, besides the genuine reversals of forked and scute, above mentioned, apparent reversals of them have been found, caused by so-called suppressors in other loci, and the same holds in the case of any character in *Drosophila* in regard to which a suitable

search is made. In fact, in mutant stocks of *Drosophila* a search for such suppressors is often unnecessary, as natural selection often works against the mutant character, so that a stock tends to undergo reversion to phenotypic normal by accumulation of mutant genes in other loci, the effects of which simulate that of reverse mutation of the gene primarily dealt with. This I found first in keeping stock of bent wings in 1914, and confirmed it, in collaborative work with Marshall (1917), by measurements made in the case of three other mutant characters—dachs, curved, and balloon. Stocks of the latter had, through processes of natural selection occurring within the laboratory cultures, accumulated more modifiers acting in the direction of the normal type in those cases in which the genes in question themselves, i.e. dachs, curved, or balloon, were kept homozygous and hence had a chance to manifest these mutant characters, than in lines in which, being always kept heterozygous, they had no chance to attain expression and so to be selected against. Real reverse mutations of the three genes in question themselves had not happened to occur, however, so that the partial reversal found was, in a more exact genetic sense, spurious.

As the numerous mutations with very small effects are usually less harmful than the large mutations, it will more usually be by the former that change in the direction of selection will proceed. And when large mutations do play their part, smaller ones will usually be selected to render the former more acceptable, i.e. to "buffer" them, to use an apt expression of Huxley's (1936). Just what constellation of smaller mutations shall become established in any given interbreeding group, however, must be largely a matter of chance—of which ones in the whole potential number happen to arise and become spread, by the process of accidental differential multiplication, either to the point of virtual fixation itself or to the point where selection can take hold and then cause their multiplication up to the point of virtual fixation. Hence we find on intercrossing different groups in which a prolonged parallel selection has occurred—as, for example, in Timoféeff-Ressovsky's (1927) experiments with the radius incompletus venation character in *Drosophila funebris*—that though the populations in question may be much alike phenotypically, they are apt to differ greatly in the genetic basis of the given character. Prof. D. M. S. Watson has called my attention to a notable example of this principle seen in crosses of cattle, in which the hybrid cows, derived from crosses between two high-grade milk strains, commonly give a much lower milk yield than the parent strains, thus illustrating the genetic diversity of the strains subjected to parallel selection. Paradoxical though it may seem, heterosis illustrates essentially the same principle, so far as the question here under discussion is concerned.

The argument put forward above with regard to parallel evolution of course applies, *mutatis mutandis*, for reversed evolution as well. That is, it is too much to expect that reverse selection shall go back over just the same path by which it came. Moreover, the longer that path has been, the more does this hold true, and the probability of exact retracement varies (inversely) more nearly as a geometrical than as an arithmetical function of the length of the path (i.e. of the number of mutational steps). And neither the sequence of steps (i.e. the permutation they represent) nor

the final collection of them (i.e. the combination they represent) will be the same in two non-intercrossing populations, still less will it be the same in one of them as in the primeval population back to which a phenotypic reversion of evolution has occurred through reversed selection. It is also to be noted that, despite the existence of a superficial resemblance in some phenotypic respects, the longer the path in question has been, the more likely are we to be able to detect differences in the developmental stages, in the physiological and chemical mechanisms, and even, on closer scrutiny, in the adult morphological characters, whereby the equivalent or similar phenotypic end-effects in the two species which we are comparing are attained.

It must further be taken into consideration that if the parallel or the reverse selection in question does not involve all the characters of the organism at once, that is, if any characters are allowed to remain without undergoing the parallel or the reverse change in the direction of the given type, their presence will change the conditions of development and of functioning of the other characters—those that are being selected—so that the latter, to attain the given phenotypic expression and type of functioning, will require a somewhat different type of development and hence, too, different genes, from those which would operate best in the case of an ideally complete condition of parallelism or reversion. This is because of the intricate interlocking both of the processes of development, and also of those of physiology, of ecology, etc.

V. THE ROLE OF INTERLOCKING AND DIFFUSION OF GENE FUNCTIONS IN HINDERING TRUE REVERSAL OF EVOLUTION

In addition to the sheer statistical improbability, amounting to an impossibility, of evolution ever arriving at the same complex genic end-result twice, whether by reverse, parallel or convergent evolution, there is the point raised in the discussion concerning relaxation of selection, that a given gene or genetic differentiation, once established, tends to become necessary in other ways than it may have been at the beginning. This will operate to cause its retention for a long time, not only in the absence of selection for the character that it primarily or originally ministered to, as was illustrated in the discussion concerning the locus of white eye in *Drosophila*, but even in the face of actual selection against that character. To be sure, some "buffers" may eventually arise—that is, genes that compensate for a change in one or more of the secondary effects of the primary gene in question—but meanwhile and perhaps for a very long time or indefinitely the primary gene will continue to operate in essentially the old way. The same holds true for combinations of genes giving rise to given structures or processes of the organism. For there is undoubtedly far more transfer of function in evolution, largely of a non-adaptive, fortuitous nature, than has commonly been realized. It is a kind of spreading out (and at the same time thinning out) of function on the part of the individual gene, whereby an embryological or physiological process or structure newly arisen by gene mutation, after becoming once established (with or without the aid of selection), later takes more and more part in the whole complex interplay of vital processes.

For still further mutations that arise are now allowed to stay if only they work in harmony with all genes that are already present, and, of these further mutations, some will naturally depend, for their proper working, on the new process or structure under consideration. Being thus finally woven, as it were, into the most intimate fabric of the organism, the once novel character can no longer be withdrawn with impunity, and may have become vitally necessary. At the same time, however, the new process which the original mutation in question had initiated may now, itself, have come to depend upon many genes, and relatively little upon the original one.

A more or less morphological illustration of the above spread of function, applying to a structure that by the present day must itself be the end-result of numerous genes working in combination, is to be found in the process which leads to gill-slit formation in vertebrates. No longer necessary in mammals for gills, it has become necessary for the formation of the parathyroid gland and other structures, although, had it not been present in the first place, analogous glands might have evolved from other primordia. Similar considerations no doubt apply, in a less morphological manner, in the case of the abolition of the hind limb girdle in whales, for it would probably have been abolished completely, if only by degenerative processes, had not the processes involved in the development of these rudiments been secondarily bound up, in some way unknown to us, in the development or maintenance of other features of the animals' economy.

The lower viability of the larvae of the vast majority of mutants of *Drosophila*, even where the mutations seem primarily to concern structures only useful to the imago, like eyes, wings and legs, shows how general is the spreading out of function of each gene, and how great the consequent interlocking of functions, and hence, conversely, how far-reaching must be the division of the genetic basis of any given vital phenomenon so that the latter comes to depend on an increasing number of genes, by a kind of genetic diffusion process, even where there may be no advantage in this subdivision in itself. As a result, evolution, even if it reverses some of its steps more or less superficially, must now find roundabout paths to circumvent an interference with vital functions, and it cannot be expected, in so doing, to retrace (in reverse) the same largely fortuitous route as that by which it has come.

The newer steps of evolution, whether they be "progressive" or, in their end-results, more nearly resemble returns to an earlier evolutionary condition, will thus tend not to disturb much the more basic reactions of development and physiology, but rather to overlay them, as it were, adding on further reactions subsequent to or in the later stages of the former. This is the genetic basis of recapitulation, which is thereby seen to have a real foundation in the kind of constitution of the organism. At the same time, however, it must be recognized that there is nothing absolute in this tendency, and that even older, more basic reactions can eventually become radically altered or abolished, by having other, supplanting reactions gradually evolved, while newer reactions may more easily become again dispensable.

Although the similarity of organs or of characters thus arrived at by apparently reverse (or parallel) evolution necessarily involves, as we have seen, a considerable diversity in the ultimate genic basis, and hence in the qualities of the original

chemical reactions whereby the rest are conditioned, nevertheless it seems rather a metaphysical quibble to contend that the similarities in such cases cannot represent "real homologies". Homologies must after all, here as elsewhere, usually be matters of degree, and these homologies may in some of these cases be just as real as homologies usually are, especially where shorter evolutionary paths, in more nearly related organisms, are involved. For even though the genic, chemical bases of the characters in question differ, in so far as they depend on mutations arisen since the two types separated, nevertheless these newer genes are working in connexion with a complex constellation of other genes which is giving largely the same kind of reaction system in the two kinds of organisms being compared. In these reaction systems the initial gene-reactions become, as it were, canalized, so as to lead to a given, limited set of structures and reactions on the upper phenotypic levels, and changes in numerous different genes may thus produce virtually the same change in embryology, morphology, physiology, and even biochemistry, as judged by the moderately analytical methods of contemporary specialists in these fields.

These points have not adequately been taken into consideration by those who contend that, while there may be reverse changes in regard to proportions and numbers of parts, these must not be counted as real reverse evolution, whereas reverse changes of organs, involving their re-establishment—which would constitute real reverse evolution—cannot occur (see e.g. Gregory, 1936). Organs also are made of parts and much of their development could often be analysed into changes of the proportions and numbers of the latter, while on the other hand the quantitative changes in bodily form, etc., may be traced to qualitative chemical differences originated by their genes. The arbitrary nature of the distinction in question becomes even clearer when we consider that the organs, like the bodily proportions, represent phenotypic features far removed from the genes themselves. Even the processes and structures on the intermediate phenotypic levels, lying below and conditioning those organs or characters that we ordinarily take note of, may be virtually the same, in effect "homologous", and yet many of the genes themselves, lying beneath the former in turn, may be different.

VI. EVER MORE PRONOUNCED IMMISCIBILITY AS AN INEVITABLE CONSEQUENCE OF NON-MIXING

There is no doubt that the process of gradual incorporation of innovations ever deeper into the whole web of necessary biological reactions goes on to a far greater extent in the intricate realm of biochemistry in general than among those more special phenomena which are characterized by distinctive morphological expressions. When this process takes place in the case of mutations affecting the complex reactions upon which the fertility of the organism depends, a population arises which tends to give sterile hybrids, or not to cross, with its ancestral or sister populations. For many innovations in the chemistry of germ-cell production that at first were virtually neutral, or were merely somewhat advantageous, must later, through subsequent mutations in other genes affecting the chemical reactions of reproduction, have become much more important or even quite necessary in the system of reactions

(so long as these latter mutated genes were acting as well). And since all genes are not equally dominant, or dominant to the degree here required, hybrids between the more recently evolved type and the old will have some of these new, now necessary reactions above referred to developed too little to interact adequately with those other new reactions, still later acquired, in the presence of which the former reactions are now necessary. Essentially the same sort of thing, occurring in the realm of reactions necessary not merely for fertility but for the life of the soma, must eventually render the new population incapable of giving viable F_1 hybrids with a population like that from which it was derived, or with another population, independently derived from the same ancestral one. The much quoted killifish of Kosswig (1929) and the lethal cereal hybrids of Hollingshead (1930) show this process in its incipience, when a new gene that gives harmonious results only in the specific genotype of a certain population (or species) and not in that of a related population (or species) still exists in a non-fixed condition in the former population.

In genetic jargon we should say that in such cases the given group had at some time come to contain a genetic "suppressor" of the harmful effect of some other mutant gene or gene combination, that had in fact not at that time come to exist yet, or at least had not come to be widespread, in that group. This potential "suppressor" might consist either of one gene or of a combination of genes, and it might have become established either by accidental multiplication, or by selection for some advantageous effect, other than the above effect itself. In most cases, however, the advantageous effect in question might equally well have been brought about by mutations in other loci, if they had happened to get a foothold, and so, even in the case of selection, it was largely a matter of chance that just this particular suppressor became established. Later, the mutation or combination of mutations that formerly would have been harmful would be able to become established too, being now rendered innocuous or possibly even helpful by action of this "suppressor". Thus the "suppressor" gene or gene combination, though at first neutral or merely an asset, would, after the establishment of the second mutation or series of mutations mentioned, have become much more important than before, or even vitally necessary, for viability or reproduction, and from then on it would play an essential part in the complex system of necessary life processes. And if crossings between this newer stock and one which in these respects was like the original were then carried out, the F_1 would show the deleterious effects of the potentially harmful mutant genes in all cases in which the "suppressors" happened not to be sufficiently dominant to counterbalance completely the heterozygous action of these other, harmful genes.¹

The important thing to note here is that these changes in the chemistry of the organism become established in a given population in a largely fortuitous manner, the selection at the time requiring only that they be not harmful, or at best that they be helpful in a given respect for a given end-result, without regard to what chemical conditions they may set up for the establishment of later changes in the chemical

¹ In the crossing of two groups descended by separate paths from the same original group, disharmony could also arise by the deleterious interaction of different mutant genes or gene systems of the two groups, that separately worked adaptively.

mechanism.¹ In one group of organisms, then, those alterations of the chemical structure and reactions which become established will differ from those in another group, even though the latter be undergoing the same type of phenotypic selection, and from that time forth the paths of chemical development of the two groups have become set in divergent directions. The two groups, if kept separated, will eventually become quite uncrossable because of the accumulation of these differences, and they will continue to diverge chemically more and more, no matter how parallel their phenotypic development may be. So too, in the case of "reverse evolution", a group once separated from some ancestral type will necessarily diverge more and more from the latter chemically, and consequently in its crossability, no matter how close to the latter it may appear to be returning, either morphologically, or in respect to its general physiological or ecological modes of reaction.

The phenotypic resemblance in the latter case must therefore be a superficial one, no matter how far-reaching it seems. But that it can be far-reaching is evident from the very definite conditions which selection often sets in regard to a whole complex of characters, for forms living a certain way of life. A case in point here is the striking resemblance in bodily appearance and manner of living between the dog and the Tasmanian (marsupial) "wolf", or between the rhinoceros and certain extinct mammals of different lineage. Another illustration is the likeness of the independently evolved eyes of the vertebrate, scallop and higher cephalopod. For changes in bodily form, proportions, pigmentation, etc. can easily be brought about by many different mutational routes and so, since optimal co-ordination under given conditions often calls for a very strict set of relationships between the parts, with little essential leeway, a very similar-seeming end-result may be attained. The two forms, however, though so resembling one another, are, as it were, built out of different materials. As before noted, however, the more nearly related they were to begin with, the deeper will the resemblance in the evolutionary products of parallelism (under which we include also convergence) or of reversion go, owing to the limitations set by the "canalization" of the developmental and physiological reactions with which they were originally provided.

At the same time, we should also recognize that the so-called parent species itself (that is, the population that remains in form and functioning much like the original type) must necessarily be passing through its own chemical transformations, though selection may long keep it very constant in form, like a fossil that undergoes an inner metamorphosis by chemical replacement of its material, while still retaining its original conformation in most minute detail. The smaller the population, or populations, of which an apparently stable species, already arrived at its phenotypic optima, consists, the faster will be this metamorphosis, in so far as it depends upon change through accidental multiplication (the "drift" of Wright) but, as above implied, there may also be a cryptic selection, attended by a chemical change that is only casually dependent upon this selection. Thus, underneath selection, as

¹ As previously mentioned, however, despite this fortuitousness, many of the genes in question may have become established through selection, because of their effects on certain morphological or physiological characters that might equally well have been subserved by other mutations, having other chemical characteristics.

it were, there must go on a continual replacement—a shifting of the chemical, the genic basis, never to return, and with it goes a particulate, never-resting diffusion of functions among the different genes.

In the more ultimate sense, then, a reversal of evolution is an impossibility, although much phenotypic reversal (even on the deeper lying planes of partially analysed ontogenetic and physiological processes) may take place, and although the individual genes often undergo reversion in a much more exact, possibly even in an accurate chemical sense. But collectively, there cannot be a real reversion of the genes, unless selection were not natural but guided by biologists far more intelligent than ourselves. And with the different genetic bases acquired in the course of the pseudo-reversion the analogous groups of organisms, similar though they may appear, may eventually arrive at very different potentials for their further evolution. Their chemical differences will be expressed in part in their non-crossibility and increasingly also in differences in the intermediate ontogenetic stages, physiological reactions, etc., whereby the more superficial phenotypic end-results are attained. Such differences, in regard to the unselected features of their structure and reactions, features the distinctive characteristics of which would normally not affect the course of the organisms' lives, will then serve as a clue, and tests for the existence of these differences will reveal the basic unlikeness of the organisms in their intimate warp and woof.

VII. SUMMARY

1. In *Drosophila* the great majority of mutations are reversible in direction, and very commonly the "reverse mutation" appears to reconstitute precisely the original gene. The mutation from the normal to the abnormal type has been found usually to be a change from a more active to a less active condition of the gene, and is hence to be regarded as constituting, itself, a reversal of the original direction of evolution. The so-called reverse mutation, in such a case, is really a mutation in the direction of past evolution. As the latter changes usually occur less readily than the former (except in the rare cases of highly mutable mutant genes), it is to be inferred that evolution proceeded contrary to the prevailing mutation pressure, and hence only by the aid of selection. Thus, with selection relaxed, a certain reverse evolution would tend to occur, so far as individual loci were concerned.

2. The reversibility of most mutations is significant in the theory of the gene and of evolution in showing, first, that most mutations are not mere losses of genes. Secondly, as Timoféeff-Ressovsky, Zimmer and Delbrück (1935) have pointed out, the fact of their fairly high reversibility indicates that most mutations involve canalized reactions in a unified gene structure. For, in the case of radiation mutations at any rate, it can be shown that activation of any one of a large number of atoms, and of gene parts, results in sensibly the same mutation; that this principle applies even to reverse mutations indicates that precisely the same gene-part as was struck in making the original mutation need not be struck again to make the reversal. This adds to the already existing evidence (Muller, 1932) that the gene mutation process involves a chain of reactions of which the primary one may even have lain outside

the gene. It is at present uncertain whether or not this process involves a breakage and linear rearrangement of the chromonema similar to that occurring in obvious gene rearrangements but much more minute. This conception, which might require a revision of older notions of distinctly delimited genes, seems however to meet with some difficulties in explaining the comparative readiness with which apparently exact reversions may be produced.

3. Considerations of mutation frequency show that a mere removal of selection for given genes would be attended by reversal of evolution, in the sense of loss of organs and of traits (e.g. pigmentation) dependent on organized reaction systems, in, geologically, a comparatively short time. In practice, there are difficulties in the way of such a stoppage of selection for given genes (or even for given alleles of them) inasmuch as there is a tendency for genes (and gene differences) to become increasingly pleiotropic in the course of evolution, through mutational transfer of functions. Stopping selection in respect to the major function of a gene, then, can only slowly, with a speed dependent on the recency with which the gene has acquired this function, lead to a genetic reversal, involving loss of this function. For such a process is now contingent upon the establishment of mutations in other genes, that accidentally happen to have the effect of taking over the secondary functions of the gene in question. Eventually, however, loss of any function must follow stoppage of selection for it, since an ever greater number of mutations must become established (both through selection for other functions, and through "drift") that happen to disturb the organized reaction system whereby the given function is carried out.

4. There can be apparent reversal of evolution with respect to given characters brought about by selection of mutations as well as by the genetic disintegration attendant upon mere removal of selection. But in neither case will the final product be genically identical or even very similar to the archetype. For the mutations of many different genes have equivalent end-effects, especially in the case of the "small mutations", which are more numerous and less harmful, and hence more apt to furnish evolutionary material than the large ones. For this reason the determination of the exact mutational path of evolution involves a large element of accident and, considered from a genic point of view, this path can never really be retraced, nor paralleled, in a second evolutionary sequence, nor can the same complex genic system be twice arrived at. The probability of the phenotypic similarity being thorough-going will depend, among other things, on the length and complexity of the path to be retraced (or paralleled), and on the extent to which the reverse (or parallel) selection applies to all features at once (as a departure in one respect will tend to influence the conditions for other features).

5. In the case of a longer, more complex path, there is an increasing role played by the complicating circumstance (mentioned in (3)) that some of the evolutionary steps have later acquired accessory functions that can no longer be dispensed with readily. At the same time their own genetic basis has spread so as to depend on an increasing number of genes, by a kind of genetic diffusion. These circumstances will often prevent even the appearance of retracement, so that an equivalent end-result (e.g. adoption of fish-like form by mammals) will obviously embody a quite different

developmental mechanism or have a demonstrably different morphological or physiological basis. There will thus be a tendency for the old gene reactions of development and of physiology to persist in the basis of the life complex and only to be overlaid, as it were, by the newer acquirements, which would tend to develop later in ontogeny (recapitulation), and this principle would apply no matter whether these newer acquirements represented progressively different stages, or more or less phenotypic reversal to an earlier stage (a superficial reversion). Nevertheless, especially in the case of shorter paths (and more closely related organisms) there should often be the possibility both of parallel and reverse evolution involving a more nearly real retracement (forwards or backwards) of steps which, though genically somewhat different, embody essentially the same reaction changes, as judged from the point of view of ordinary embryology, physiology and morphology. For here the steps have not yet become so indispensable; moreover, the thousands of primary gene reactions are necessarily canalized into certain definite channels, that limit the possible effects of their change, as viewed by these methods, which still deal with characters standing relatively far from the gene itself. To some extent, then, reversal, as well as parallelism in evolution, may be "real", and to deny the homologies of the resultant forms is to make an arbitrary metaphysical distinction, created to suit the point to be proved.

6. The complex systems of chemical reactions upon which fertility and viability depend become changed by numerous mutations, differing in different populations, that become established in, geologically, a very short time. Some of these mutations, though first indifferent or only an asset, finally become necessary, through the later establishment of other mutations, which without them would be detrimental to fertility or viability. Thereafter, crossing between one of the populations in question and one like the original (or one likewise evolved from the latter) will result in hybrids that are sterile or inviable, owing to the action of these harmful mutant genes, inadequately balanced by the ones that had made them tolerable. Two groups of organisms which are not ordinarily allowed to cross with one another will thus automatically become increasingly immiscible, and their genic, chemical paths of evolution will diverge more and more. This will occur even in cases where their evolution is, from the phenotypic standpoint, strikingly parallel (owing to similar selective conditions and similar developmental and physiological bases for change), or where one of the groups undergoes a striking appearance of reversion towards the other, and even though, in the case of more closely related groups, the parallelism or reversion may involve physiological and ontogenetic processes lying on a relatively deep plane of analysis. There must also be a hidden shift in the chemical, genic basis of a population which, phenotypically, remains relatively constant. But although these deeper-lying genic changes may for a long time remain cryptic, they will eventually find more and more expression in "unnecessary" features of the life processes, discoverable by the chemist, the physiologist, the embryologist, or the morphologist, and an ever more different basis will be laid conditioning the future evolutionary possibilities.

The author acknowledges with thanks the aid of Dr Sarah Bedichek Pipkin in the preparation of the manuscript for this article.

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ADDENDUM

Since the submission of the present article, the proof has kindly been sent to me of a valuable paper by Bernhard Rensch, "Typen der Artbildung", which is being published in this Journal¹ while the present article is in press. It is shown in Rensch's paper that the evolutionary principles discovered by the systematist and the paleontologist are in harmony with the conceptions of modern genetics concerning the basic role, in the process of formation of races, species and higher categories, of fortuitously arising mutations (the expression of which is, however, conditioned by the whole genetic, developmental and physiological system), accidental multiplication of (some) mutants, and selection. Parallel or convergent evolution is interpreted by Rensch, as in the present paper, as resulting from (1) similarity of the genetic (and consequently of the physiological) basis, which is hence subject to similar types and combinations of changes, and from (2) similarity of the conditions of selection. Concerning reverse evolution (which is only referred to briefly) the paper of Rensch, like the present paper, points out that, although its elementary steps—reverse mutations—certainly do occur, nevertheless reverse evolution as such cannot occur, because of (1) the multiplicity of mutations and (2) the fact that a real identity of selective influences is precluded by the complexity of the circumstances which condition the selection.

¹ *Biological Reviews*, vol. 14, 1939, p. 180.

THE MOLECULAR CHAIN STRUCTURE OF CELLULOSE AND ITS BOTANICAL SIGNIFICANCE¹

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(Received 18 October 1938)

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I. INTRODUCTION

It is becoming increasingly evident that a knowledge of the detailed structure of the cell wall will eventually throw light on many problems in botany, and will prove to be of paramount importance to the developmental anatomist, to the cytologist, and to the physiologist. Several reviews of the study are already available, discussing the general aspects (Seifriz, 1934; Anderson, 1935; Frey-Wyssling, 1935) or more specialized branches (Sisson, 1932-3, 1933-4; Stamm, 1936; see also Meyer & Mark, 1930; Norman, 1933). It is not proposed here to give any comprehensive account of investigations already ably covered in these publications. The principal aim is to present, in as concise and simple a manner as possible, some idea of the principles underlying chemical and physical approaches to the problems concerned.

The importance of considerations of cellulose structure in the solution of some botanical problems has been recognized since the time of Nägeli. Thus, to quote only a few instances, we find Nägeli himself (*loc. cit.*) attempting explanations of deposition and growth, both of the cell wall and of starch grains, in terms of his micellar hypothesis; numerous contemporary and later investigators explaining the form and growth processes of the wall in terms of somewhat similar conceptions;

¹ Based on a series of lectures delivered before the Honours School in the Department of Botany, University of Leeds.

showed that the normal form of glucose has a six-carbon atom ring structure; and further, that there are two stereo-isomeric forms of this sugar. On account of their resemblance to pyran he called these pyranose sugars. This occurrence of two stereo-isomers is readily intelligible in terms of the cyclic model. In this six-ringed form, for example, the carbinol group and the —OH attached to carbon 1 may lie either on the same side of the ring or on opposite sides, somewhat as in Fig. 1*a*. Now the β -form is characterized by its ready conversion to laevo-glucosan (Fig. 1*b*), so that the aldehydic hydroxyl group in this form must lie on the same side of the ring as the carbinol group. On such grounds the formulae represented in Fig. 1*a* are given to α -glucose, and to β -glucose. The five-ringed modification of glucose is also known in two stereo-isomeric forms whose resemblance to furan has caused them to be termed α - and β -furanose forms.

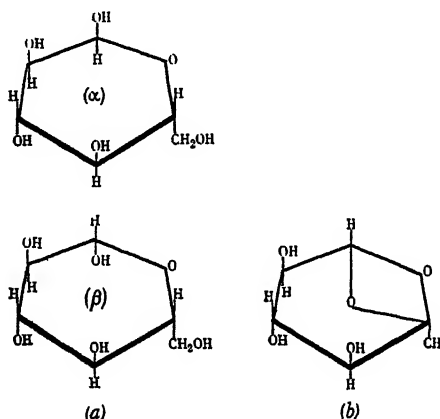


Fig. 1. (a) Stereo-chemical formulae for the pyranose forms of α -glucose (upper) and β -glucose (lower) (modified from Haworth). In α -glucose the OH attached to carbon 1 is on the side of the ring opposite to the OH of the carbinol group. In β -glucose the two groups are on the same side. (b) Stereo-chemical formula for laevoglucosan. This is clearly derivable from the β form of glucose only.

Now the reducing power of cellobiose is only half the value one would expect from its constituent glucose residues. Undoubtedly the aldehydic group of one sugar is masked by linkage to a non-reducing —OH of the other, i.e. the linkage in cellobiose is glucosidic.

The nature of the sugars involved has been investigated by Haworth, Hirst, Irvine, Long, Peat and Plant, Haworth, Long and Plant (see Haworth, 1929) and others. Briefly, the method of attack has been as follows. There are eight available hydroxyl groups in cellobiose, all of which may be protected by methylation. This methylation leads, of course, to loss of reducing power. On carefully controlled hydrolysis, however, reducing power reappears, and it is evident that one —OH group must have developed in each hexose, only one of which is reducing. It remains to identify the resulting methyl hexoses. One of these is readily shown to be 2, 3, 4, 6-tetramethyl glucose; and this could only have arisen from the non-reducing component (for each component already possesses one —OH , as a result

of hydrolysis, and the reducing group readily loses its methyl group during this process) from a pyranose sugar (see Fig. 2*a, b*).

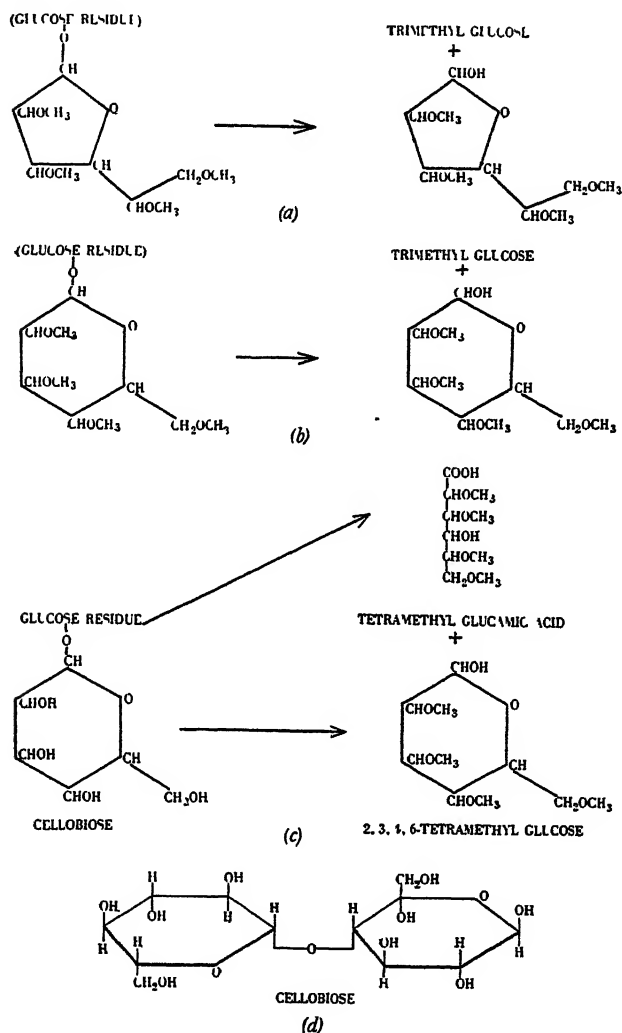


Fig. 2. (a) The result of methylation and hydrolysis of cellobiose, on the assumption that the non-reducing glucose component is in the furanose form. Carbon 4 has no attached —OH group and therefore, cannot carry an acetyl radicle. The resultant compound is therefore 2, 3, 5, 6-tetramethyl glucose. (b) Similar to (a) except that the non-reducing component is in the pyranose form. Here, therefore, 2, 3, 4, 6-tetramethyl glucose is produced. (c) The mild oxidation of cellobiose followed by methylation and hydrolysis. The reducing component gives rise to a tetramethyl glucamic acid, with an —OH on carbon 4; the position of the —OH necessarily implies that the reducing component of cellobiose must be attached to the non-reducing component at carbon 4. Since this carbon in the furanose form of glucose has no attached —OH group (Fig. 2*a*) linkage could not occur here in this form of the sugar. Hence the reducing component also must be in the pyranose form. (d) The structure of cellobiose as formulated by Haworth. Both components consist of pyranose forms of glucose and both are in the β -modification. Linkage occurs between carbon 1 of the non-reducing component and carbon 4 of the reducing. The chemical evidence indicates that cellulose is built up of chains of such cellobiose units, linked regularly by 1:4 linkage, with the elimination of water.

The remaining half of the products—trimethyl glucose—could have arisen either from a furanose or a pyranose form. That it represents a methylated pyranose sugar was made clear from the products resulting from mild alkaline oxidation of cellobiose. The aldehydic group is oxidized to a carboxyl with consequent opening of the ring. Methylation and hydrolysis of the resulting acid gave, as before, a 2, 3, 4, 6-tetramethyl glucose (which again was derived from the non-reducing component) and a tetramethyl glucamic acid (Fig. 2*c*). In this latter, only the fourth carbon is devoid of a methoxy group, and this must inevitably be the group to which the aldehydic group of the first sugar was attached. Further, since the fourth carbon in the furanose form of glucose has no attached —OH group (Fig. 2*a*) linkage could not occur here. Hence the second sugar, too, is a pyranose form and the two sugars are linked through carbon atoms 1 and 4. The question as to the precise spatial relationships of these two pyranose sugars may be answered in terms of the behaviour of cellobiose to enzymes. Enzymes of the nature of α -glucosidases (e.g. diastase) will not attack cellobiose, whereas it is readily hydrolysable by β -glucosidases (e.g. emulsin). If we may accept this specificity of enzymes for definite groupings, and the evidence seems all in its favour, then there can be no doubt that both components of cellobiose are in the β -pyranose form. Confirmation of this result has also been given by more orthodox chemical means (Haworth, 1929).

(b) Cellulose

Thus we may assume cellobiose to have the structural formula ascribed to it by Haworth (Fig. 2*d*), of which an independent derivation has been provided by Zemplen (1926). The conclusion has already been reached that cellulose consists of an aggregate of such cellobiose units. While it is to X-ray analysis (see later) we owe our present knowledge of the kind of aggregation, evidence of a purely chemical nature is not without interest and significance. It was early suggested (Freudenberg, 1921) that the constituent glucose residues in cellulose are linked into long molecular chains, and this view, in spite of much early criticism (Karrer, 1921; Hess, 1924; Pringsheim, 1926), has proved substantially correct. Strong evidence was obtained by the separation from cellulose, as degradation products, of the so-called "oligosaccharides". To Bertrand & Benoist (1923) is due the first isolation of a trisaccharide, though a similar substance was later separated by other workers (Irvine & Robertson, 1926; Ost, 1926). Some years later Willstatter & Zechmeister (1929) isolated substances they believe to be cellotriose and cellotetrose, a result which was later confirmed by Zechmeister & Toth (1931), who also added a new member to the series—cellohexose. It was found that the properties of these sugars changed progressively as the series was ascended from glucose; and it has become clear, by methods analogous to those used above, which need not be discussed here, that the linkage between glucose residues is universally of the 1:4 type described for cellobiose itself (Haworth *et al.* 1931). This has been confirmed in an elegant manner by Freudenberg *et al.* (1932), using the optical rotations of oligosaccharide and cellulose solutions.

Finally, it must be observed that if cellulose consists of long chains of glucose residues then one of the terminal glucose units of each chain must be unique in the sense that it contains four hydroxyl groups capable of ether formation. As early as 1914 (Denham & Woodhouse) traces were found of a substance resembling tetramethyl glucose, but subsequent failures to detect such a substance led either to a denial of the long-chain theory (Irvine & Hirst, 1923), or to the belief that the chains are extremely long (Freudenberg & Braun, 1928). To Haworth & Machemer (1932) we owe the final proof that tetramethyl glucose is obtained as a hydrolytic product of trimethyl cellulose, with its necessary corollary that cellulose is a series of molecular chains. On the basis of these determinations, the length of the molecular chains was estimated as some 200 glucose units. As will appear later, there is some question as to the precise meaning which is to be attached to this figure; but in spite of the possibility of degradation during treatment, leading to a smaller chain length, this final determination of Haworth & Machemer's is of the greatest interest and value.

2. EVIDENCE FROM X-RAY ANALYSIS

During the years immediately following the experimental trial, by Friedrich & Knipping, of Laue's suggestion that X-rays should be diffracted by crystals, the new tool thus placed in the hands of the crystallographer was employed over a wide range of substances; for it was early realized that the diffraction pattern thus obtained from a crystal is characteristic of that crystal, and enables some deductions to be made concerning its structure. With some of the simpler inorganic compounds almost immediate success was attained, though many of the substances of a more complex type still remain incompletely determined. Among these latter we may place cellulose. During more recent years some modification of what we may, perhaps, call the classical model of cellulose has been suggested (Meyer, 1937; Sauter, 1937), but until confirmation has been given it is unnecessary to say much about these recent developments; especially as they are such as to have little bearing on the present considerations.

The atoms in a crystal may be considered to lie in a series of planes which can reflect X-rays at certain angles given by

$$2d \sin \theta = n\lambda,$$

where n is an integer, λ is the wave-length of the X-rays, d is the spacing between a set of planes, and θ is the angle of incidence (see Fig. 3). This is the formula developed by Bragg and is known as the Bragg Law. If, therefore, the value of n is known (the "order" of the reflexion), d may be calculated, since λ is known and θ may be measured (θ is commonly rather small). The precise character of the "reflected" beam depends not only on the distance apart of the molecular layers, but also on their number. The position of *strongest* reflexion is the same whatever the number of layers; but with a small number there is a considerable range over which detectable reflexion occurs, decreasing as the number of reflecting planes increases. Only when there are many planes, regularly spaced, is there anything like a sharp

reflexion (compare Figs. 4*a*, *b*). Hence, if such a reflexion is observed, we may be sure that it corresponds to a series of many planes accurately spaced a measurable distance apart.

The experimental procedure in the investigation of the structure of plant fibres is illustrated in Fig. 5*a*. In general, a bundle of fibres is used, though single cells have occasionally been investigated (Patterson, 1928; Preston, 1934). A series of diffraction spots are obtained, much as in Fig. 5*a*, and for any such spot on the developed photograph the corresponding interplanar spacing, d , may be calculated.

Since a fibre is essentially a hollow cylinder, the X-ray photograph obtained from it is the same as that which would be given by a single longitudinal strip of the wall continuously rotated; the diagram resembles, in fact, that obtained from a crystal rotated about one of its axes, the so-called rotation diagram. Hence it is clear that

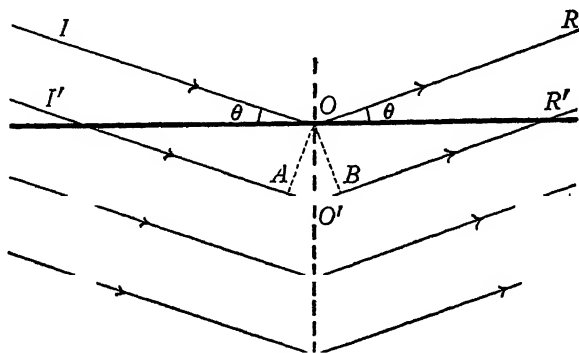


Fig. 3. Diagram to illustrate the Bragg Law. The horizontal black lines represent planes of atoms. $IIOA$, incident beam of X-rays. $RR'OB$, reflected beam of X-rays. θ , angle of reflexion. OO' , normal to the planes of atoms. OA and OB are perpendicular to $I'O'$ and $R'O'$ respectively.

in such fibres as we are concerned with here (ramie, hemp, etc.) one of the axes of the cellulose "crystals" is oriented approximately parallel to the length of the fibre. The distance between corresponding points in this direction may readily be calculated as 10.3 \AA . ($1 \text{ \AA} = 10^{-8} \text{ cm.}$) (e.g. Sponsler & Dore, 1926; Polanyi, 1921; Herzog & Jancke, 1928; Meyer & Mark, 1930), and we may take this as the magnitude of one of the periodicities. Determination of the remaining two parameters of the three-dimensional pattern is not so easy. The first complete interpretation given by Polanyi has been confirmed by later work (Herzog & Jancke, 1928; Meyer & Mark, 1930), and the dimensions of the unit cell are generally given as

$$\begin{aligned} a &= 8.3 \text{ \AA}, & c &= 7.9 \text{ \AA}, \\ b &= 10.3 \text{ \AA}, & \beta &= 84^\circ. \end{aligned}$$

A somewhat different suggestion was put forward by Sponsler & Dore, whose figures, however, represent exactly the same structure. Until recently, the figures given here have been accepted as crystallographically more representative; recent work, however, shows a tendency to return to Sponsler's scheme (Sauter, 1937).

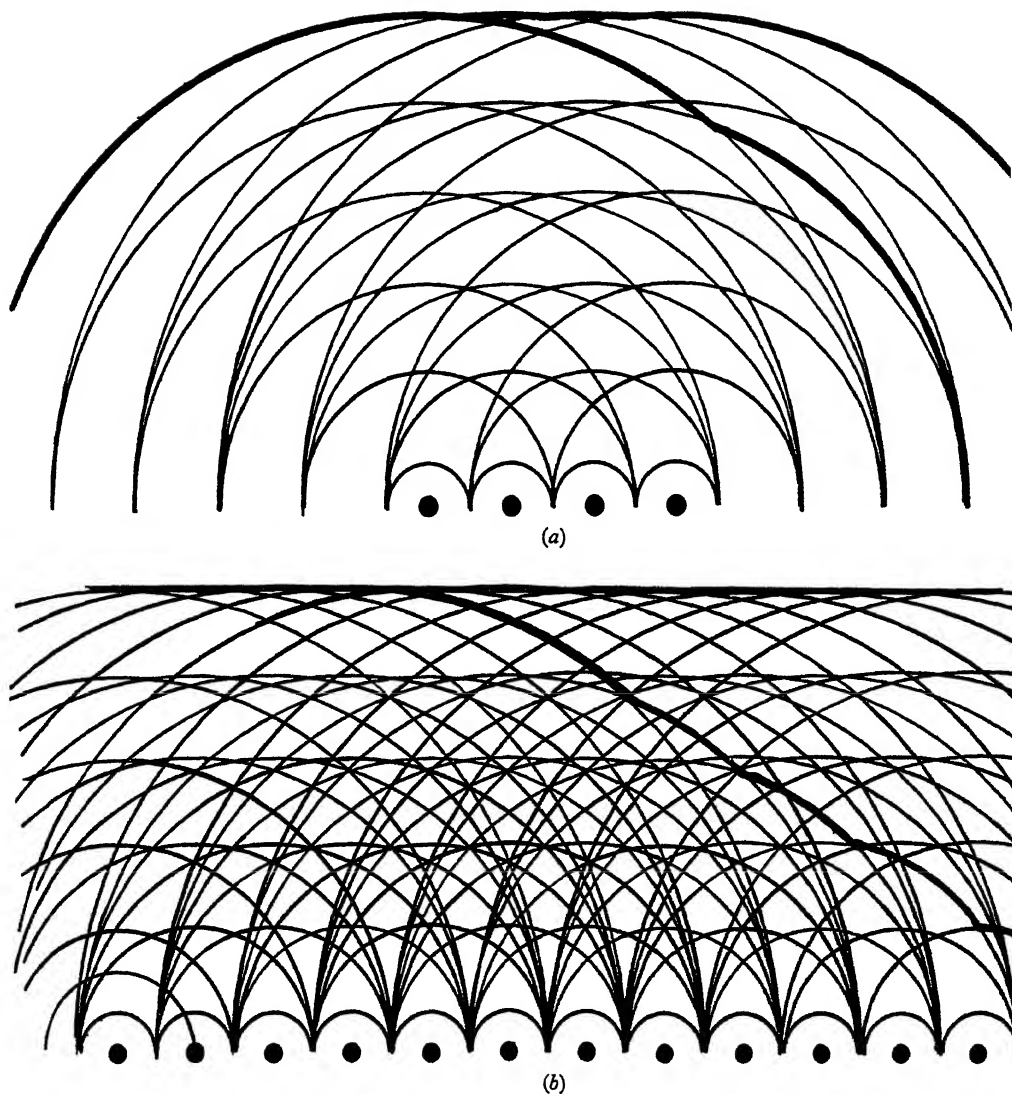


Fig. 4. Diagrams to illustrate the effect of the number of diffracting points on the character of the diffracted beams. In both figures the radiation is conceived as travelling parallel to the surface of the paper, from below upwards. The diffracting points are indicated by black circles, and the waves diffracted from each such point as circles drawn with these points as centres. (a) Many diffraction points. The constituent, circular wave fronts result in the transmission of the incident beam in the original direction. At the same time a wave front is reflected at a considerable angle to the incident wave front. Both are emphasized in the diagram by thickened lines (the phenomenon is best observed by holding the page on the level of the eyes and glancing along the horizontal thickened line. If the page is now rotated about a vertical line it will be observed that tangents to a series of circles become collinear when glancing along the sloping thickened line). Both wave fronts are sensibly straight and the records on a photographic plate would be clearly defined spots. (b) Few diffraction points. Two wave fronts are delineated as in (a). Here, however, both have curved margins. The record on a photographic plate would therefore be two diffused images, rather denser towards the centre and gradually decreasing in density towards the outside.

The above figures may, however, be reached by a more simple, if less logical, procedure. It is clear (Sponsler, 1931) that the problem would be more easy of solution if the fibre be cut open longitudinally and rolled out into a flat lamina. Such a process is, of course, impracticable, but corresponding flat plates of cellulose are now available. The cells of the green alga *Valonia*, for instance, are sufficiently large to allow the removal of a single piece of wall (Sponsler, 1930, 1931; Preston,

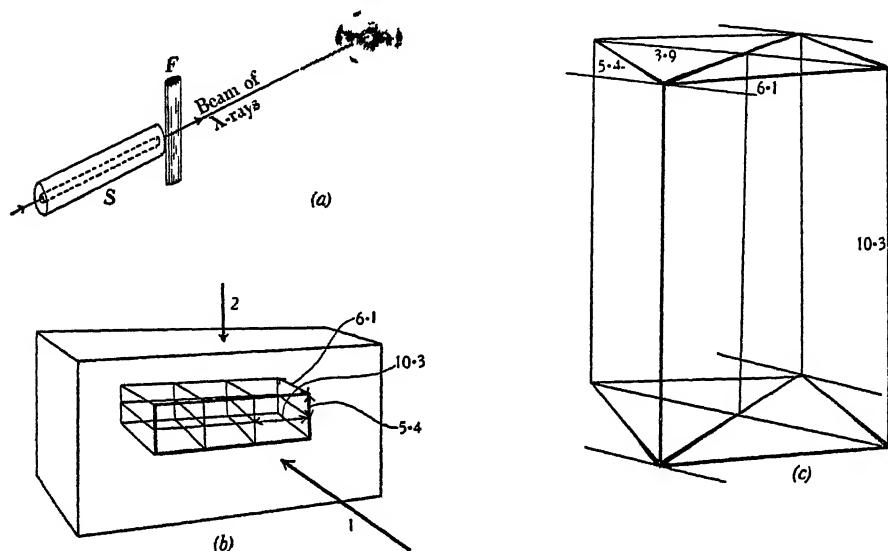


Fig. 5. (a) The fibre photograph of cellulose. A beam of X-rays impinges on a slit system, *S*, consisting of a hole, generally some 0.5 mm. diameter, drilled through a cylinder of material impervious to X-rays. The fine beam issuing from the slit passes through a bundle of parallel fibres and is prevented from reaching the photographic plate (with consequent blackening) by a lead cup (which causes the white "shadow" in the centre of the photograph). Some of the radiation is reflected by molecular planes in the fibres, and these reflected beams are recorded as spots or arcs on the diagram. The extensive outer arcs, on each side of the centre along the horizontal row, correspond to planes of 3.9 Å. spacing; the inner arcs on the same line are fusions of the 6.1 and 5.4 Å. reflexions. Note that the arcs on the upper and lower sides of the diagram are much narrower radially than those on the right and left. (b) The position of various molecular planes in a plate of cellulose in which the chains run horizontally. (c) The "unit cell" of cellulose suggested by Sponsler. Although this particular unit cell is not widely accepted, it does represent the relationship between the various spacings given in the figure.

1931; Astbury *et al.* 1932; Preston & Astbury, 1937); the so-called bacterial or B-cellulose may be obtained in the form of plates; and, finally, in the tests of the tunicates we have, potentially, flat plates of a substance *tunicin*, identical with cellulose. Investigation of all these specimens has been made. The direction of the 10.3 Å. spacing is readily detected as before. Now, if the X-ray beam is directed perpendicularly to the plate (1, Fig. 5*b*) there is no reflexion corresponding to planes spaced 6.1 Å. apart; but there is a strong reflexion at 5.4 Å. On the other hand, if the beam is directed as in 2, Fig. 5*b*, the conditions are reversed—there is

now a strong reflexion at 6.1 Å. but none at 5.4 Å. By rotating the plate around the horizontal axis, the angle between the position of strongest reflexion for the planes of 5.4 Å. spacing and that of the planes of 6.1 Å. spacing may be determined. It turns out to be almost 90°. The planes of 6.1 Å. incidentally appear to be approximately parallel to the surface of the plate. By similar methods the planes of 3.9 Å. spacing may be shown occupying a position intermediate to those of 6.1 and 5.4 Å. (Fig. 5c). The values 5.4 and 6.1 Å. were suggested by Sponser (1925-6) for the parameters c and a , and 88° for the angle β (Fig. 6a), but this clearly involves an assumption. On the other hand, Meyer & Mark (1930), for instance, assumed these figures to represent, not the dimensions of the unit cell, but spacings between other, definite, planes in the structure. They determined the unit cell given above by a method whose consideration demands considerable mathematical skill and which need not be described. The unit cell thus suggested would seem to be the only one from which the remainder of the diffraction spots on the photograph may be derived (and, of course, there are many more than have been considered here). This is, of course, the crucial test—any proposed structure must account for the positions and intensities of all the diffraction spots. The unit cell most widely accepted is represented in Fig. 6b; it remains to determine its contents.

The first suggestion was due to Sponser & Dore (1926). They noted that the dimensions of the glucose molecule (whose spatial formula had already been proposed by Haworth as a six-atom ring), calculated on the basis of known interatomic distances, was such that two residues linked by an oxygen bridge had a length of 10.3 Å. They therefore postulated that these molecules fit in the unit cell as this relation would suggest. From such considerations they proposed the structure illustrated in Fig. 6a and since, by definition, repetition of such a unit cell through space will reproduce the crystal structure, the molecular chain hypothesis of cellulose structure was readily derived. To Sponser is, in fact, due the first clear evidence that such molecular chains do exist. The fact that he considered his diagrams best explained by a 1 : 1, 4 : 4 alternate linkage does not detract from the importance of his work. Later determinations, by other workers already mentioned, confirmed, however, the 1 : 4 linkage proposed for cellobiose by the chemists (Fig. 6b). The idea of molecular chains of glucose thus largely rests on relatively simple considerations. The unit cell represents, as it were, the "building stone" of the crystal, and the length of a molecule of cellobiose may be fitted exactly with the 10.3 Å. dimension. In laying these building stones one upon the other we are obviously constructing a molecular chain. As the building proceeds, the carbon atoms are found to lie in definite planes and these may be correlated with the various X-ray reflexions obtained.

Thus the results of X-ray analysis confirms the work of organic chemistry; and supplements it by demonstrating that the molecular chains of cellulose lie parallel to each other in a very exact manner (linked together, presumably by van der Waals forces) and pointing in a direction which can readily be determined. In X-ray analysis we have, in fact, the only reliable means of determining cellulose chain direction. Again, even in the question of the dimensions of the cellulose chain, this

method confirms and supplements those of the organic chemists. It is clear from Fig. 4 that the breadth of an X-ray diffraction spot is governed by the *number* of planes from which it is derived. On the basis of breadth measurements of such spots, Hengstenberg & Mark (1928) have ascribed to the chain a length greater than 600 Å., which compares favourably with the value derived by Haworth

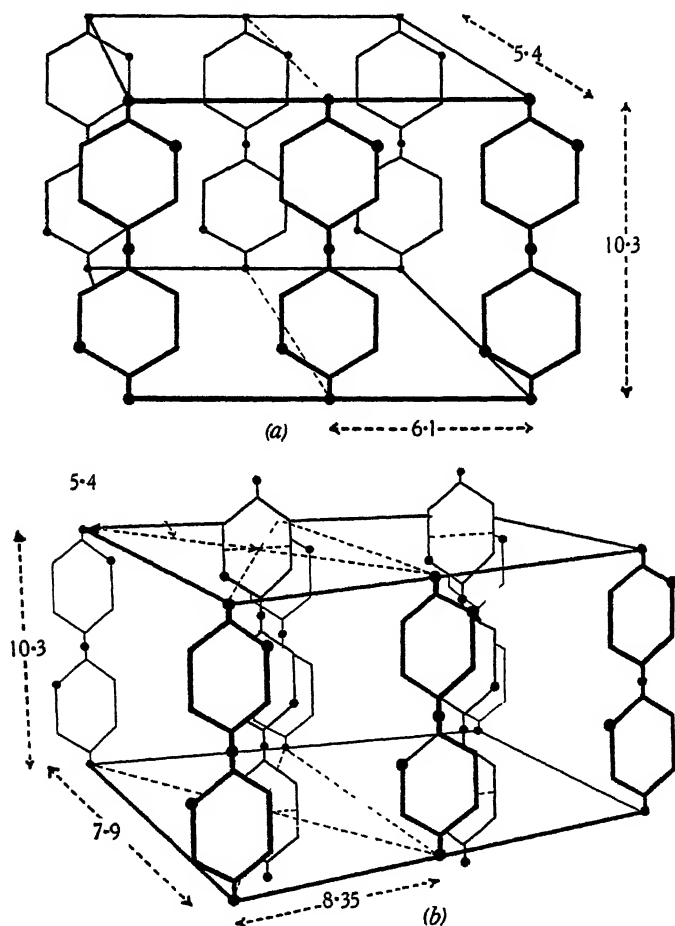


Fig. 6. (a) The space lattice of cellulose as first proposed by Sponsler & Dore. (b) The space lattice derived by Meyer & Mark, now widely accepted as crystallographically correct. In both figures the dimensions are given in Angstrom units. The black circles represent oxygen atoms. Hydrogen atoms and the carbinol group on carbon 5 are omitted.

(200 glucose residues = $200 \times 5.15 = 1030$ Å.). Here again, however, there is some uncertainty, consideration of which is perhaps better postponed to a later section.

It is to be noted that, in spite of the contention of some workers in other fields (e.g. Bailey & Kerr, 1935), the presence of cellulose can be detected, and even the direction of its molecular chains be determined, even though other substances are present. This is particularly true in the case of those plant cells containing incrusting substances which, on account of the failure of their molecules to conform to a

regular pattern, do not give an X-ray diagram. Thus the presence of lignin, for example, has no apparent effect on the X-ray diagram obtained with some pericyclic and phloem fibres, and the removal even of xylan causes no material disturbance (Astbury *et al.* 1935*b*).

3. VISCOSITY MEASUREMENTS

Of several other methods of investigation which have been used in an attempt to demonstrate the existence of molecular chains in cellulose, and to determine their length, the technique of viscosity determinations as evolved by Staudinger is the only one which need be discussed here. The problem of the structure of cellulose is not, of course, an isolated one; it is bound up intimately with the whole problem of the nature of polymerization, and it is from this point of view that Staudinger's work is of special interest. Through the use of synthetic polymers of increasing chain length, Staudinger found that the length of the chain, as derived from X-ray diagrams, is a linear function of the degree of polymerization and of the molecular weight as determined cryoscopically (Staudinger, 1926, 1929; Staudinger *et al.* 1927). In some of the series studied, variation of chain length was sufficiently wide to enable the relation between physical properties and chain length to be studied. Polymers of short-chain length are generally powders which dissolve readily to give solutions of low viscosity, while those of long-chain length are tough, elastic solids which, if they swell at all, yield highly viscous solutions. On such grounds, Staudinger was able to support the conception of cellulose as a linear polymer. But further than this, he was able to derive an empirical equation connecting the molecular weight of his synthetic polymers with the viscosity of their solutions; and though the validity of his equations could be established only on polymers of low molecular weight (short-chain length), he claims that the constants obtained by the use of these substances can be used for determining the chain length of a higher polymer of the same series. Several anomalies have arisen from the rigid application of such viscosity laws to cellulose and its derivatives, and several workers have shown that the equation does not hold for the higher polymers of some series (Kraemer & v. Natta, 1933; Meyer & van der Wyk, 1935). Nevertheless, the figures derived by Staudinger for cellulose from various sources, based on equations applicable to highly degraded cellulose and its derivatives, are of considerable interest; for purified ramie, for instance, he gives a chain length of 1000, and for cotton 750, glucose residues. These figures are considerably in excess of that given by Haworth, and of the lower limit suggested by Hengstenberg & Mark. While it is questionable whether in higher polymers chain length is the only important factor influencing viscosity (the degree of cohesion between neighbouring chains, for example, may clearly play a part) it is not impossible to harmonize these results (p. 300).

III. MICELLAR STRUCTURE

The results of X-ray analysis suggest not only that cellulose exists in the form of long molecular chains, but that the chains are grouped into bundles—the so-called micelles (p. 297). This is, of course, no new conception for it originated with Nāgeli,

who was led to propose his Micellar Hypothesis on the basis of swelling phenomena and optical behaviour. That the cellulose chains are not, in fact, so continuous and uniformly arranged as unbroken repetition of the unit cell would require, is shown clearly by tensile strength determinations. The tensile strength of cotton hairs, for instance, is about 20 kg./mm.² and of Irish flax 100 kg./mm.² or over. Now the strength of the C—C link may be calculated from the known chemical energy (3×10^{-12} erg.) and the known distance apart of the constituent carbon atoms. It is found that a force of some 3×10^{-10} kg. is necessary to rupture such a primary valence bond. If we take the cross-sectional area of a molecular chain of cellulose as 30 Å.², then an area 1 mm.² contains 3×10^{12} chains. The theoretical tensile strength of cellulose, therefore, if the chains are as long as the fibre, is of the order of 900 kg./mm.² Some kind of discontinuity must clearly occur in the structural arrangement of cellulose such as is implied in the micellar hypothesis.

In comparatively recent times several lines of investigation have lent support to this conclusion. Apart from the method of X-ray analysis already mentioned, perhaps the most outstanding are those involving double refraction phenomena, in which the material is examined under a polarizing microscope. It is not proposed here to give any detailed account of the physical principles underlying the technique—they may be readily obtained from text-books on the subject (e.g. Ambronn & Frey, 1926; Winchell, 1931; Johannsen, 1918)—though they demand some brief consideration in order that the value of the method may properly be appreciated. Plane sections of crystals can transmit light vibrating only in two directions, which are at right angles to each other and generally bear some relation to the crystal axes. They may be characterized by the difference between their optical properties in the two different directions. Particularly important is the difference in refractive index, and it is on this basis that the term “double refraction” or “birefringence” is defined. If the refractive indices in the two directions are n_γ and n_α ($n_\gamma > n_\alpha$), then

$$\text{double refraction} = (n_\gamma - n_\alpha).$$

Further, since the two directions of vibration, in such a section of anisotropic material, correspond to these different refractive indices, then the two vibrations which are “in step” as they first develop in the plate, are out of step on leaving it by an amount equal to

$$(n_\gamma - n_\alpha) d,$$

where d is the thickness of the plate. This is defined as the “path difference”, and can readily be measured; hence if d is known ($n_\gamma - n_\alpha$) may be calculated. Alternatively n_γ and n_α may be measured independently (by an immersion method (Frey, 1926 *a*; Ambronn & Frey, 1926; Preston, 1935)), giving much more valuable and instructive results.

Cellulose is characteristically doubly refractive and the double refraction may readily be determined. Here we have, then, further proof of the crystalline nature of this substance. Only under certain conditions do the extinction positions bear any

relation to the direction of the cellulose chains, i.e. when the object under examination consists of chains lying in one direction only. One of the positions (corresponding to n_y , and often called the *major extinction position*) is then strictly parallel to the direction of the cellulose chains. This may clearly be demonstrated in many fibres (Herzog & Jancke, 1928), and has been proved for other cases (Preston, 1934; Preston & Astbury, 1937). This is, of course, exactly what theoretical considerations would demand for such a structure, and the double refraction of cellulose is clearly a reflexion of its crystalline nature.

At the same time it has been realized for many years that a different kind of double refraction may be encountered—the so-called form double refraction which was used originally by Hofmeister (see Nägeli) in an unsuccessful attempt to discredit the micellar hypothesis. If a substance consists, for instance, of long, narrow, isotropic cylinders of refractive index n_1 imbedded in an isotropic medium of refractive index n_2 , then the composite body will have different properties in different directions (Fig. 7a). If the cylinders are narrow compared with the wavelength of light, the body will be optically anisotropic, for the refractive indices n_y and n_x will be different. This type of anisotropy can readily be recognized for the value of the double refraction depends on n_2 ; and as n_2 approaches the value n_1 the double refraction decreases until when $n_2 = n_1$ we have an optically homogeneous substance which is therefore isotropic ($n_y - n_x = 0$). A theoretical consideration of the problem has been made by Wiener (1912), who showed that the double refraction of such a body is related to the refractive indices of its components by the equation

$$n_y - n_x = \frac{\delta_1 \delta_2 (n_1^2 - n_2^2)^2}{(\delta_1 + 1) n_2^2 + \delta_2 n_1^2},$$

where δ_1 is the relative proportion of the substance of refractive index n_1 , and $\delta_2 (= 1 - \delta_1)$ is the relative proportion of the n_2 component. Some part of the double refraction of cellulose must be due to this phenomenon if micelles are present. The structural double refraction of cellulose is generally so high, however, that the slight change expected with change in imbedding medium may be masked. Some evidence of such an effect has occasionally been observed in fibres and collenchyma (Möhrling, 1922; van Iterson, 1933), and parenchyma (Bonner, 1936).

More convincing is the work of Frey (1926a) on the silica matrix of grass haulms. After the organic matter has been removed from such an organ, in the usual way, a so-called skeleton of silica remains, whose double refraction varies with the imbedding medium (Fig. 7b). Clearly there are spaces in the silica which may be filled with liquid. It has been found impossible accurately to test the formulae given by Wiener, since δ_1 and δ_2 are difficult to ascertain with sufficient accuracy. The shape of the curve, and especially the position of the minimum, is not so precise as could be desired, especially when the method is applied to substances capable of swelling. For it is essential, in using the method, that the material should not be swollen (δ_1 and δ_2 constant). Yet if any liquid enters the intermicellar spaces there must, theoretically, be a slight swelling. Further, the liquid in the intermicellar

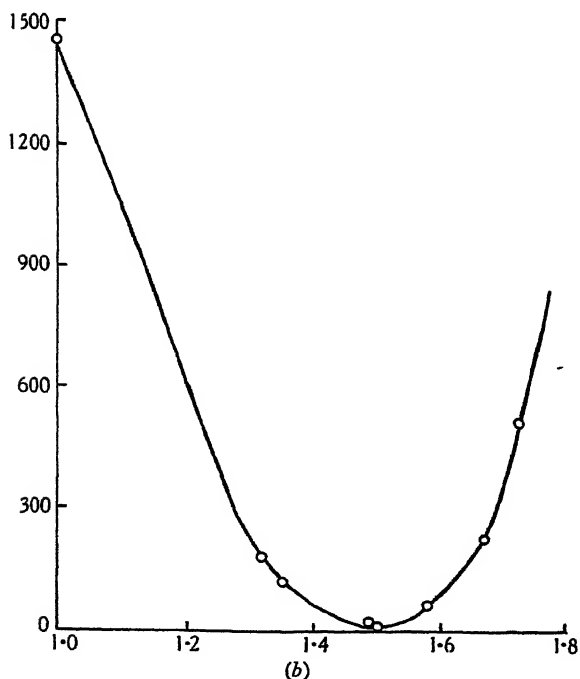
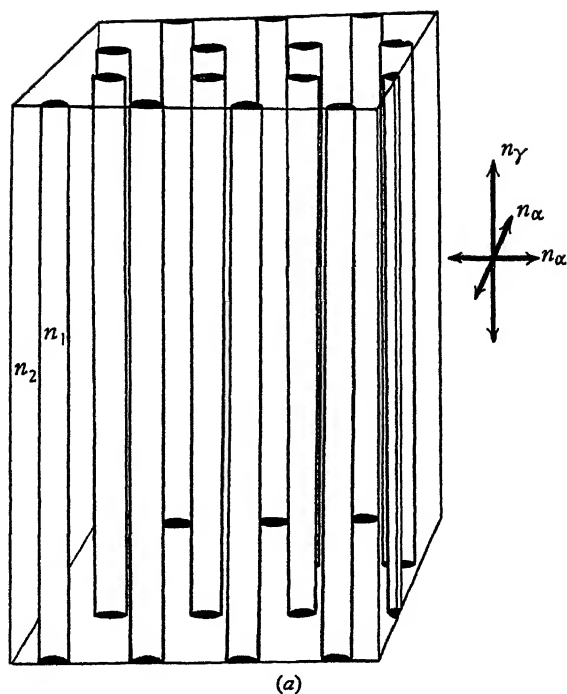


Fig. 7. (a) A "mixed body" consisting of a series of long, narrow isotropic rods imbedded in an isotropic medium (after Frey-Wyssling). n_γ , n_α , the principal refractive indices of the mixed body; n_1 , the refractive index of the rods; n_2 , the refractive index of the imbedding medium. (b) Curve showing the relation of the double refraction to the refractive index of the liquid of imbibition, for the silica skeleton of a grass haulm. Ordinate: path difference in Angstrom units. Abscissa: refractive

spaces comes under the influence of surface forces and is in a condition somewhat different from that of the free liquid outside the fibre. It certainly has a higher density, a lower vapour pressure, its refractive index is certainly different, and it may even acquire anisotropy of its own by orientation on the micellar surfaces (Castle, 1936*b*). The refractive index of the free liquid is, in fact, no accurate measure of that of the liquid in the fibre. This source of error is considerably increased when dealing with mixtures of liquids, for one may be adsorbed preferentially. In general terms, however, the observed phenomena are in harmony with the idea that the cellulose in the cell wall is not a homogeneous entity. On such grounds it may be suggested that cellulose exists in the form of micelles in the wall.

In certain instances the optical properties of the wall may be used to define the directions of the micelles. It is always found that the major extinction position of the wall, as seen in section, lies parallel to the wall surface; which must of necessity mean that the micelles are similarly oriented. In face view, the major extinction position may be inclined to the length of the cell at an angle varying with individual cells. In fibres the angle is generally small, in tracheids of medium size, and in vessels generally larger (Fig. 8). With some cell types (e.g. some parenchyma, sieve tubes) the wall is apparently isotropic. Only when the wall consists of one set of cellulose chains may the conclusion be reached that the micelles are oriented parallel to the major extinction position as observed in this view (e.g. some tracheids (Preston, 1934), some vessels (Preston, 1938), single layers of the *Valonia* wall (Preston & Astbury, 1937), probably parenchyma cells of oat coleoptiles (Preston, 1938)). With the annular, spiral, or reticulate thickenings of protoxylem vessels (Fig. 8) and the tertiary thickenings of metaxylem vessels and tracheids (Frey, 1926*b*; Frey-Wyssling, 1935), with bars traversing the perforations of vessels (e.g. the multi-perforate cross-walls of *Helianthus annuus* (Smith, 1935; Preston, 1935)) the major extinction position and presumably the micellar direction, lies parallel to the length of the thickenings, and is independent of that of the underlying wall when such is present. In the border of bordered pits, it always lies parallel to the nearest edge of the border (Frey, 1926; Preston, 1938; Bailey & Vestal, 1937; Scarth *et al.* 1929).

Consideration of polarized light phenomena give, however, no exact measure of micelle size. We have seen that the method of X-ray analysis can yield a figure for the length of the molecular chains; the same is true for the lateral dimensions of the micelle. It is a very common feature of X-ray photographs of cellulose fibres that the spots or arcs corresponding to planes of molecules parallel to the fibre length (e.g. 3.9, 5.4, 6.1 Å.) are much more diffuse than those corresponding to planes perpendicular to this length. An explanation of this effect is readily deduced in terms of Fig. 4. The crystalline fraction of the cellulose clearly consists of particles which are long and narrow—in other words cellulose is micellar in structure. We may picture the micelle as more than 500 Å. long and some 50 Å. in diameter. It was first pointed out by Sponsler (1931), however, that this conclusion is not contained in the facts. The logical deduction is that only within this region are the molecular planes so arranged as to be capable of reflexion. Sponsler, working on the green alga *Valonia*, suggested that the so-called micelles are actually not rods but

plates; and that the plates are curved so that only in a narrow median strip are the molecular planes in reflecting positions. This criticism is valid, and the model

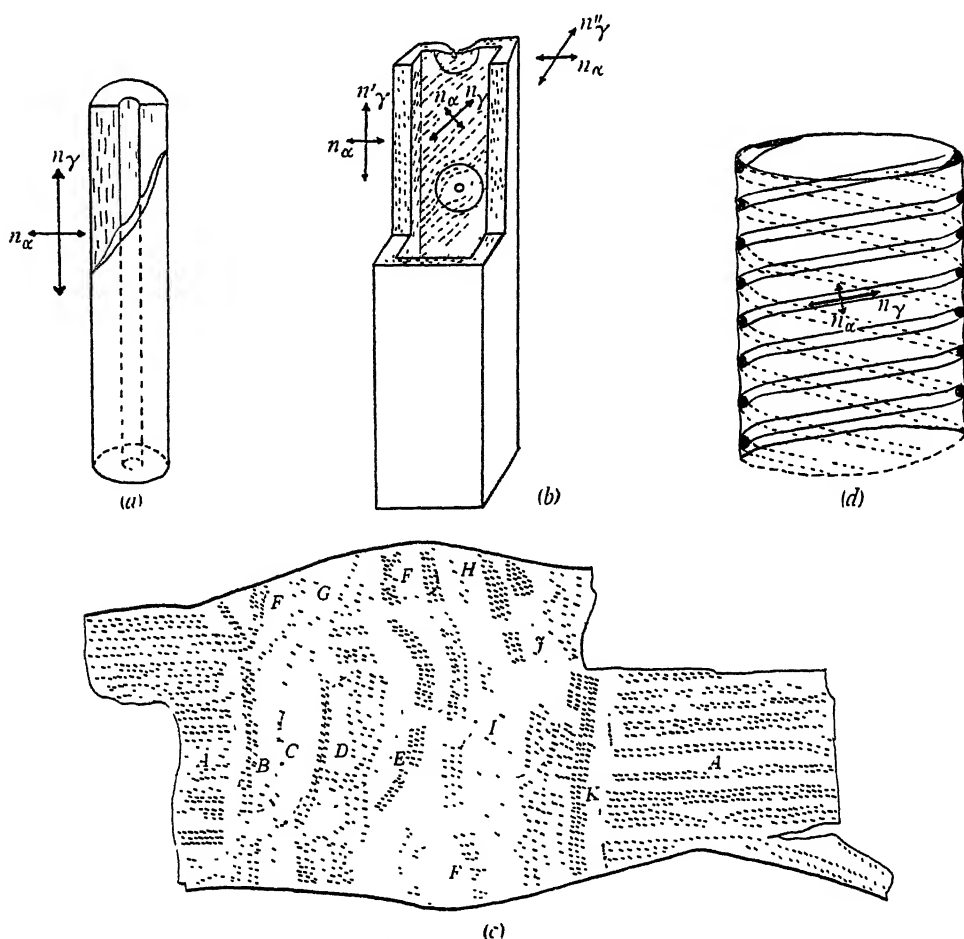


Fig. 8. (a) and (b). The optical properties of a fibre (a), and a tracheid (b). The direction of the major extinction positions is indicated by n_γ . The direction of the cellulose chains in the bulk of the wall is shown by broken lines, modified in sections of the wall as demanded by conditions of perspective. (c) The run of the cellulose chains in the secondary wall of a vessel element in *Fraxinus americana*. The lines in the diagram represent the position of the pits and the approximate directions of their slit mouths. These correspond also to the directions of the cellulose chains, which are inclined to the horizontal, in the various areas, at angles as follows: A, 23; B, 22; C, 34; D, 24; E, 17; F, 44; G, 32; H, 59; I, 39; J, 19. (d) The spiral thickening of a protoxylem vessel. The major extinction position and the cellulose chain direction in the thickening are parallel to the spiral winding.

of the micelle which is generally accepted to-day does differ from classical conceptions. The modification proposed by Sponsler has received little attention, though in *Valonia* the diffraction spots corresponding to planes of 3.9, 5.4 and 6.1 Å. spacing are peculiarly sharp (Sponsler, 1930, 1931; Astbury *et al.* 1932; Preston & Astbury, 1937).

On the other hand, there is much evidence of an indirect nature which lends support to the classical micellar hypothesis. Dye absorption experiments, for instance, have led to the conception of large internal surfaces. Paneth & Radu (1924), using methylene blue, estimated the internal surface of cellulose preparations at 10^4 – 10^5 cm.²/g. This is probably too low, on account of the size of the dye molecule and of preferential absorption of water, and the value of 10^7 cm.²/g. obtained from gas adsorption experiments (Kälberer & Schuster, 1927) is probably more accurate. The theoretical figure, corresponding to the micelle size given by Hengstenberg & Mark, is still higher (7×10^7 cm.²/g.) (Meyer & Mark, 1930), though the discrepancy may be due to a very slow diffusion of gas into the smaller pores. Phenomena of swelling offer another method of attack. It has been shown by Katz (1924) that when a fibre swells in water the X-ray diagram is unaltered; nor does a ring appear on the diagram, due to water in bulk, until the fibre is soaked. Clearly, then, the initial swelling is due neither to penetration of the space lattice nor to absorption into large capillary spaces; the water must be intermicellar.

Various attempts have been made to measure the size of intermicellar spaces in dried fibres. The relatively large difference between the apparent specific weight (as determined from the weight and dimensions of fibre bundles) and the real specific weight (as determined by helium displacement) has been used for the purpose. Thus Balls (1928) found the apparent specific weight for cotton hairs to be 1.27 and the real 1.55, which indicates a void volume of some 20%, while Clegg & Harland (1923) reached a figure of some 32–41 % in the case of bast fibres. These figures would indicate the dimensions of the intermicellar spaces as about 10–20 % of those of the micelle. The methods used in the determination of the apparent specific weight are clearly rather inaccurate and these figures can hardly be exact. Somewhat higher figures are obtained by Berkmann *et al.* (1926) who obtained X-ray diagrams of bast fibres impregnated with metallic silver. This diagram is that typical of cellulose, upon which is superposed a series of rings corresponding to particles of silver arranged at random. The fact that the impregnated fibres show the optical phenomenon known as dichroism (see e.g. Ambronn & Frey, 1926; Frey-Wyssling, 1935) would presumably indicate that the silver particles lie in intermicellar spaces. Their size may be calculated (again from the breadth of the rings in the diagram) and they appear to be of the order of size of the micelle diameter. Larger figures still have been obtained by Frey-Wyssling (1936), but these clearly do not represent the state of the intact spaces. Frey-Wyssling assumes that his figures refer, not to intermicellar spaces but to larger pores in the wall. The validity of any of these impregnation methods is open to question, since the crystallization forces involved in building up a silver particle may be sufficiently great to force the micelles apart; and it has, in fact, been shown (Bailey & Vestal, 1937) that microscopically visible crystals may be grown in the wall, though the technique involved is rather drastic.

In recent years the classical micelle theory has fallen into disrepute. As pointed out by Peirce (1930) the broadening of the X-ray spots is equally well explained by a structure consisting of bundles of parallel molecular chains separated by

regions of more or less random orientation. Similar ideas have been put forward by Astbury & Woods (1932), Miles (1933), Neale (1933), Frey-Wyssling (1936), Freudenberg (1932) and others. This proposed amendment to the micelle theory is in harmony with the bulk of the evidence quoted above, and explains some otherwise anomalous phenomena such as the failure of cell walls to collapse after the removal of incrusting substances (e.g. Bonner, 1936). Undoubtedly this relatively new conception does harmonize the views of Staudinger and of Hengstenberg & Mark concerning chain length. It is conceivable that the treatment used by Haworth has involved degradation of cellulose by attack of the comparatively "free" chains in the "intermicellar spaces". In the following pages the term "micelle" is used as interpreted in this later definition.

IV. MICROSCOPICALLY VISIBLE FEATURES AND OPTICAL PROPERTIES

During the past 100 years repeated attempts have been made to confirm the existence in the wall of particles larger than micelles—bodies which may be called micelle aggregates. Thus Dippel (1879), Schmitz (1880), Krabbe (1887) and Strasburger (1898) explained the striations in the wall of phloem fibre, previously observed by Nägeli, in terms of contact faces between adjacent "screw bands" in intimate contact. Wiesner (1892), again, was led to the interesting speculation that the wall is composed of "dermatosomes", separated by layers of "some protein or its derivative", a residue of the original protoplast. These minute bodies he further considered to be aggregated into fibrils (giving striations) and finally to wall layers. In spite of the fact that the majority of the secondary cell walls in plants fail to show any sign of protein in appreciable amounts, this suggestion of Wiesner's must retain some interest on account of the more recent work of Farr & Eckerson (1934). These authors called attention to the presence of small spherical or ellipsoidal particles, embedded in the cytoplasm adjacent to certain walls in some growing cells (e.g. young cotton hairs, to which the bulk of the evidence refers, cross-walls of *Spirogyra*, walls of *Oedogonium* and *Valonia*, etc.). These particles are stated to stain blue with aqueous iodine followed by strong sulphuric acid, but not with iodine alone, and to be doubly refractive with refractive indices 1.565 and 1.530 (measured after the removal of an ensheathing pectin envelope)—properties which are taken to imply a cellulosic nature. Together with this goes the fact that partial hydrolysis of the wall by HCl causes it to fall apart into fusiform bodies (Farr & Sisson, 1934; Ritter¹) and finally into minute spherical or ellipsoidal particles. These latter are claimed by the former authors to be identical with the particles in protoplasm; they stain with iodine and sulphuric acid, and have the same refractive indices. The protoplasmic particles are often aggregated into chains, and the hypothesis of Farr & Eckerson is that they actually are embodied in the wall as units of structure. This is quite revolutionary, for previous conceptions of wall deposition involve some kind of

¹ Reported in an undated reprint of the Paper Industry.

pseudo-crystallization of molecular chains, or of glucose molecules, on the existing wall (see e.g. Sponsler, 1929). It is difficult, however, to accept the hypothesis as it stands. Certainly the criticism of Hess *et al.* (1936) that the wall of young cotton hair (whose cytoplasm contains the particles) fails to give the X-ray diagram of cellulose, is no longer valid in view of the contradictory results of Sisson (1937). Other lines of argument are, however, still significant. The refractive indices of the particles in the protoplasm are widely different from those given by Frey-Wyssling (1935, p. 34) (1.596 and 1.525) for purified cotton hairs. This discrepancy may perhaps be explained by a failure completely to purify the particles but, at the least, the figures fail to offer convincing proof of the presence of cellulose. Again, the fact that primary walls fail to fall apart after removal of the pectic compounds (either by 2 hr. boiling with 10% HCl followed by 2 hr. in boiling 10% NaOH, for parenchyma cells of oat coleoptiles (Bonner, 1936), or by boiling for hours in dilute oxalic acid followed by a similar treatment with dilute ammonium oxalate, for young cotton hairs (Anderson & Kerr, 1938)) would necessitate some change in the relation of the particles to each other in the wall itself. Against this line of argument may be set the observation that the thickness of a single-wall layer in the secondary wall of the cotton hair, and the thickness of visible markings on the primary wall of the same plant (Anderson & Kerr, 1938) are less than that of a particle. Finally, the further statement that Schweizer's reagent attacks only the so-called "pectin" envelopes of the particles, casts considerable doubt upon their nature. On the one hand, the implication that cellulose itself is insoluble in this reagent would, if substantiated, necessitate a complete reinvestigation of the physical chemistry of cellulose. On the other, treatment with Schweizer's reagent of a wall consisting chiefly of cellulose and pectin can be shown to result in removal of the cellulose, leaving the pectic compounds as an undissolved, amorphous mass (Bonner, 1936).

The particle hypothesis has been strongly attacked by Anderson & Kerr (1938) in a recent paper. These authors claim that the protoplasmic particles, in young cotton hairs, stain with strong iodine solutions alone and that the stainable portion of the particles is readily removed, either by treatment with diastase or hot 0.05 *N* HCl. They conclude that the particles are merely plastids containing starch.

If the particles do finally prove to be constituted as suggested by Farr & Eckerson, then some of the apparent contradictions on the botanical side may, perhaps, be not inexplicable. There is abundant evidence from plasmolytic experiments and growth studies that the relation between the protoplast and the wall may be quite different for primary and secondary walls. It may be significant that the particles appear to be found only in regions of cell extension or of new wall deposition; the particles may be effective in building up the primary wall while secondary layers are deposited by other means.

The spherical or ellipsoidal shape itself of the particles observed by Farr and by Ritter offers, of course, no evidence against their being constituted of cellulose chains; for the shape of such minute bodies may well be controlled by surface forces rather than by the forces of "crystallization" involved in the laying side by side of cellulose chains. It is certainly not impossible that the particles actually deposited

during wall formation may undergo partial "condensation" upon each other. Partial hydrolysis of the wall will then yield small particles which again become rounded off through the agency of surface forces.

Attention has often been called to the development, during the swelling of secondary walls, of particles of a somewhat higher order of magnitude generally in the form of long, thread-like bodies termed "fibrils". These have been described by Crüger (1854) and Ritter (footnote, p. 300) for wood fibres, Reimers (1922) for pericyclic fibres of several plants (see also Steinbrinck, 1927; Herzog, 1910; and Herzog & Jancke, 1928), by Balls (1922) and Dischendorfer (1925) for cotton hairs, and also by several investigators for algae (van Itersen, 1933; Preston & Astbury, 1937). It is quite clear, in some cases at least, that the fibrils visible in swollen material are in some way related to striations observable in the intact wall (van Itersen, 1933; Preston & Astbury, 1937). Their diameter appears to be of the order of 0.4μ in the unswollen condition (Balls, 1922), and they undoubtedly consist of micelles arranged parallel to their length. According to Freudenberg and his co-workers they are embedded in lignin, which is certainly inter- rather than intramolecular (Frey, 1928; Astbury *et al.* 1935 *b*). The appearance of fibrils consequent upon swelling or disintegration of the wall may signify nothing more than lines of physical or chemical weakness; it is clearly no guarantee of the presence of fibrils in the intact walls, a point which has been emphasized by the recent work of Bailey & Kerr (1935). These authors have shown that the walls of xylem elements commonly consist of interpenetrating matrices, chiefly of cellulose and lignin, grading down to the limits of microscopic visibility. They suggest that the so-called fibrils are merely dissected fragments of such a network.

Further support for this argument is already contained in the work of Ritter, and of Farr and her co-workers, which shows that fibrils may be further disintegrated into "fusiform bodies" and finally into small spherical or ellipsoidal particles. These latter are still aggregate particles (consisting of micelles (Anderson & Kerr, 1938)) for they are dichroic.¹ It is probable that more drastic treatment would result in a further degradation even of these minute particles themselves. The term "fibril" as applied to the intact wall is, in fact, much better used in the same non-committal sense as the term "micelle" and to indicate at most the presence of lines of weakness.

Finally, we reach the microscopically visible layering of the wall. In general the wall of a plant cell may be divided roughly into three regions—the middle lamella, the primary wall, and the secondary layer. In the past these terms have been used rather loosely and an attempt to clarify the nomenclature has been made by Kerr & Bailey (1934). They suggest that the term *middle lamella* is best confined to the isotropic zone which may be detected surrounding the cell as the outermost layer. To the wall of the cell in the meristematic condition is ascribed the name *primary wall*, and any layers deposited after the cell has left this meristematic condition are termed "secondary". These latter, therefore, include all those late-formed layers, spirals, etc., which have been called "tertiary" by other workers. This is a classi-

¹ For a discussion of the meaning of dichroism in relation to structure see Frey (1927).

fication which will undoubtedly have the approval of the majority of botanists and is founded on clear cytological and ontogenetic evidence.

The *middle lamella* in meristematic tissues commonly consists of pectin, and although much evidence has been adduced by Harlow (1932), Ritter (1925, 1930), Schorger (1926) and others that this substance disappears during the differentiation of woody tissues, or at least becomes transformed into lignin, it is probably retained throughout unchanged (Kerr & Bailey, 1934). The primary wall clearly contains cellulose (Tupper-Carey & Priestley, 1923; Priestley & Tupper-Carey, 1922; Kerr & Bailey, 1934; Anderson & Kerr, 1938) which fails to give a characteristic X-ray diagram (Hess *et al.* 1936) until certain wax-like substances are removed (Sisson, 1937). The presence of a wax-like substance, marking the presence of cellulose, has led several investigators to assume that the primary wall is typically non-cellulosic (Balls & Hancock, 1922; Sakostschikof & Korscheniowsky, 1932; Wergin, 1936), though this view is no longer tenable after the work of Sisson. The primary wall is faintly anisotropic, a property which is sometimes partly, but not wholly, due to the presence of the *primärsubstanz* of Wergin (Wuhrmann & Meyer, 1937), and, contrary to generally accepted ideas, is rather thick in fresh material (Kerr & Bailey, 1934). The chemical nature of the primary wall has recently been discussed by Bonner (1936).

The secondary wall is often much more complex in structure. It is typically composed of two or more lamellae, varying in optical properties (Bailey & Kerr, 1935; Freudenberg, 1932; Frey-Wyssling, 1935; Anderson, 1927), the variation often being connected with changes in chemical constitution (Van Wisselingh, 1924; Anderson, 1927; Scarth *et al.* 1929). Although much of the lignin in woody tissues is to be found in the middle lamella (Harlow, 1932; Kerr & Bailey, 1934), the lamellae of the secondary wall are generally lignified to varying degrees (Scarth *et al.* 1929; Harlow, 1932; Bailey & Kerr, 1935). Bailey & Kerr have further brought out the fact that besides this concentric arrangement of lamellae, one inside the other, the walls of woody elements also show a radio-helical structure in the swollen condition (in the untreated condition in the case of xylem elements of the tropical species *Siparuna bifida*). This is particularly striking in the case of tropical gymnosperms, but is also to be found in many species of the temperate zone. Here undoubtedly we have a fact of considerable importance, though as yet quite unintelligible. On the basis of their observations of the optical properties of tracheid walls, Bailey & Kerr and Freudenberg have put forward certain ideas concerning the structure of the tracheid. The former authors, for instance, observe that in transverse section the secondary wall of the tracheid often consists of three layers (five to many-layered types may be observed, but we will confine our attention to the simplest). Between crossed nicols, the inner and outer layers are bright, and separated by a central darker layer. Variation in wall thickness is due mainly to variation in the thickness of the central layer, which is therefore more evident in summer than in spring tracheids. The authors suggest that in the outer and inner layers the micelles run transversely, whereas in the central layer they are more nearly parallel to the length of the cell. Freudenberg suggests a somewhat similar structure from analogous

observations. It would seem, however, that this represents only one of a series of possible explanations of the observed phenomena; and one which fails to be supported by other evidence which will be discussed elsewhere.

Evidence obtained from swollen material, quoted in support of the same proposed structure, can hardly be regarded as satisfactory. Several authors (Ritter, 1930; Ritter & Chidester, 1928; Scarth *et al.* 1929; Freudenberg & Dürr, 1932) have shown that in swollen wood fibres and tracheids the wall is composed of

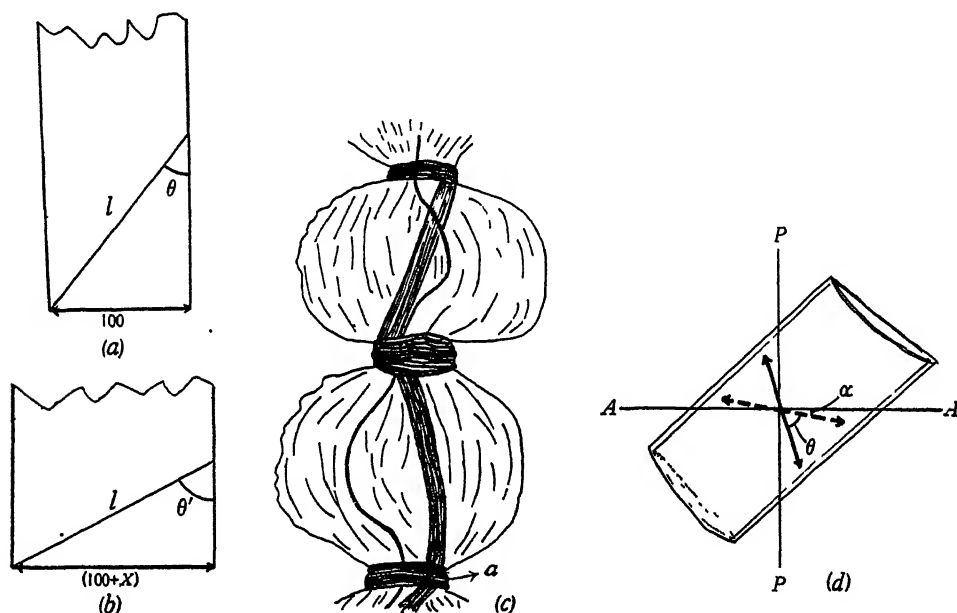


Fig. 9. (a) Diagrammatic representation of a cell cut open longitudinally and laid out flat. The periphery of the cell is taken to be 100 units. θ = angle of cellulose chains to the vertical; l = length of spiral winding corresponding to one complete turn. (b) The same cell as in (a) after a lateral swelling of $X\%$. θ' = angle of inclination of cellulose chains in the swollen cell. (c) A hemp fibre swollen in Schweizer's reagent (after von Höhnelt, from Frey-Wyssling, 1935). a = the ring- and spiral-formed remnants of the primary wall. (d) Part of a cell wound with a single molecular spiral. The continuous arrow indicates the direction of the major extinction position of the upper wall, and the broken arrow that of the lower wall. θ is the angle between them. For further explanation see text.

fibrils running almost transversely in outer lamellae and more or less longitudinally in inner lamellae. This may clearly be an artefact. If, for instance, a cell consisting of only one set of cellulose chains making an angle θ to the vertical (Fig. 9a) is swollen transversely by $X\%$, and the new direction of the cellulose chains is θ' , (Fig. 9b), then clearly

$$\frac{\sin \theta'}{\sin \theta} = 1 + \frac{X}{100},$$

if the length of the chains remains unaltered. If $\theta = 30^\circ$, then a swelling of only 100% would reduce θ' to 90° . Further, if an inner layer fails to swell, as seems to be the case with tracheids (Bailey & Kerr, 1935), the degree of flattening of the spiral will be greater the farther the point of observation is removed from the non-swelling

layer, i.e. outer layers will tend to be flatter. The phenomenon will clearly be still more pronounced if the cell "balloons" on swelling. This "ballooning", or swelling of the wall in localized regions, has been observed by several investigators. Attention has been directed to it in late years chiefly by Lüdtkke (1928, 1932), although identical phenomena had been described by Nägeli and von Höhnelt. The figure given by the latter author (Fig. 9c) is particularly interesting, since it is in harmony with the suggestion that the transverse orientation of fibrils in the outer layers of the wall is probably due to a flattening of previously inclined fibrils, emphasized by rupture of the wall in regions of pronounced local swellings.

On such peculiar swelling phenomena Lüdtkke has based his theory of a so-called *Fremdhauptsystem*. In explanation of the phenomena he assumes that there occurs in the fibre wall a non-cellulosic constituent which separates the various layers and surrounds each fibril. This in itself is no novel idea, since it is clearly recognized by several investigators that the cellulose fibril in the cell wall is characteristically sheathed in lignin. The revolutionary features of Lüdtkke's hypothesis are the relatively slight swelling of the *Fremdhauptsystem* and the occurrence of cross-walls of this substance which, if they occur, must be double. The hypothesis explains the production of balloons, and the failure of a section of wood to show cellulose reactions (because the cut is always made between the cross-walls). Further, it offers a ready explanation of the "chemical sectioning" of fibres (Wiesner, 1886; Searle, 1924; Velaney & Searle, 1930). The theory does not, however, fit in with some recognized facts and has led to some controversy. Van Iterson (1933) has clearly demonstrated the existence of fibrils much longer than the distance between the hypothetical cross-walls of Lüdtkke, and that certain swelling phenomena used by Lüdtkke in support of his theory are shown equally well by pure cellulose (e.g. sheets of cellophane).

It seems difficult at present to attempt any explanation of these swelling phenomena in terms of the structure of an intact cell. The one fact which is clear is that the multiplicity of lamellae, varying in reaction to swelling agents, which swelling technique thus reveals, makes it impossible to derive any exact information from swollen material. The variation in micellar direction is certainly by no means so extreme as observation of swollen material would suggest.

Observation of the intact wall shows, however, that certain types of cell undoubtedly possess a crossed fibrillar structure, particularly the cells of certain algae. In the case of *Valonia* (Preston & Astbury, 1937) the wall consists of some thirty layers alternating in cellulose direction. Even layers are built up of one set of chains, while in the odd layers the chains make an angle of some 80° with the first set. This structure is correlated with the appearance of two sets of striations in the wall which lie strictly parallel to the chain directions. This is also true of *Cladophora* (Preston & Astbury, 1939) and of *Chaetomorpha* (Nicolai & Frey-Wyssling, 1938), and the appearance of crossed striations suggests its application to some other cells (e.g. parenchyma of dahlia tubers, fibres of *Vinca minor*). It is interesting that in *Valonia* one set of cellulose chains forms a slow spiral round the cell while the other forms meridians uniting the two "poles" of the spiral. Although the vesicle is

coenocytic, from the point of view of the wall, it may be regarded as a single cell, whatever its morphological and physiological features may demand.

V. WALL DEPOSITION AND GROWTH

The evidence thus accumulated concerning the organization of cellulose in the wall refers almost exclusively to the secondary layer. While such considerations of the mature wall are of extreme importance, it is only in terms of the relation of the structure, thus revealed, with that of the primary wall that the full significance of wall structure is to be appreciated by botanists. As yet, however, there is no single case in which the structure of the primary wall has been worked out in sufficient detail. The difficulties in the way of its investigation are chiefly those involved in the detection of weak double refractions, for the primary wall is generally almost isotropic in face view. Apparent absence of anisotropy is not, however, to be taken as a sign of complete absence of cellulose, for this is clearly present (p. 303); a more probable explanation is to be sought in the presence of large amounts of isotropic substances, such as the polyuronides, which separate the micelles rather widely. Some primary walls do, however, exhibit a double refraction sufficiently high to allow the determination of extinction positions, and of these the parenchyma of the oat coleoptile forms a most interesting example. All the cells of the coleoptile are present at an early stage, and elongation of this organ proceeds solely by elongation of cells already present (Tetley & Priestley, 1927; Avery & Burkholder, 1936). Roughly speaking, the longer the coleoptile, the longer the cells. According to Bonner (1936) the cellulose chains of such parenchyma run transversely round the cell and their direction remains unaltered during cell expansion. Unfortunately the evidence upon which these conclusions are based is quite inconclusive. The observations made by Bonner were carried out under the polarizing microscope and refer primarily to the direction of the major extinction position. No mention is made of any attempt to dissect the cells so that presumably the determinations were made on intact cells. Now the extinction positions of a double wall cannot give any idea of those of the individual walls; the fact that the major extinction position of the double wall is transverse, implies only that the corresponding position of each single wall is inclined to the transverse direction at an angle less than 45° . This is a point of some importance and is worthy of further consideration. If the cellulose chains in the individual walls make an angle of θ° to each other (Fig. 10), if the walls be examined between crossed nicols, and the incident polarized light be represented by

$$p = a \sin wt,$$

then the light transmitted by the analyser is

$$I = a^2 \{ \sin 2\theta \sin 2(2\alpha - \theta) \sin^2 \frac{1}{2}\delta + \sin 2\alpha \cos^2 \theta \sin 2(\alpha - \theta) \sin^2 \delta \},$$

where α is the angle between one set of chains and the direction of vibration of the incident light, and δ is the path difference in each wall. Thus if $\alpha = \frac{1}{2}\theta$, $(90 + \frac{1}{2}\theta)$, etc., the intensity is a minimum, i.e. one extinction position is transverse (this is the

major if $\theta < 90^\circ$). Bonner's observations, in other words, are no proof of the existence of transverse cellulose chains. On the contrary, when the cell is dissected so that a single wall is observed, the major extinction position is generally strongly inclined (Preston, 1938). *It is clearly fundamental in any determination of wall structure to observe single walls.* The evidence for a failure of the chains to change in orientation during cell expansion is based on even less substantial evidence; for here half coleoptiles are used. Actually walls are clearly wound with a molecular spiral whose inclination changes with change in cell dimension, and the whole of Bonner's results are readily explicable on these lines (Preston, 1938).

On the basis of this work of Bonner's and of evidence from other lines of investigation (Oort & Roelefsen, 1932; Castle, 1936 *a* and *b*, 1937, 1938) suggestions have already been put forward as to the mechanism underlying wall deposition and growth (Castle, 1937). These suggestions originate from the fact, well recognized in engineering practice, that in a hollow cylinder of homogeneous material, whose contents are under pressure, the tension in the cylinder wall is twice as great transversely as longitudinally (so that, for instance, a cylinder of compressed gas bursts by splitting like a pea pod rather than by blowing off the end). This explains the otherwise anomalous fact that, under pressure, a cylindrical cell, such as the sporangiophore of *Phycomyces*, splits longitudinally even though the major extinction position is oblique. Castle suggests that the transverse orientation of cellulose chains in the primary wall is due to this greater transverse stress. Clearly, however, much of the evidence in favour of this view is removed by the demonstrable fact that Bonner's interpretation is incorrect, though the idea hardly seems feasible on theoretical grounds alone. In *Phycomyces*, as pointed out by Castle himself (1936*c*), the growth zone of the sporangiophore is not cylindrical in shape, but rather approaches the hemispherical (and this is true of all apically growing forms). There is little reason to expect a considerably higher stress in the transverse plane. Certainly stress is important in the sense that it causes a strain, leading to a *change* in orientation which would not occur in the absence of the stress (Preston, 1934), but it is difficult to conceive of an *initiation* of orientation by stress alone. Furthermore, in *Cladophora*, which grows apically, the wall structure changes from layer to layer much as it does in *Valonia*, yet the growing region retains the same shape throughout and there is no reason to expect any change in the distribution of stress.

The essential features of this hypothesis recall an earlier suggestion of van Iterson (1936) based on the work of Denham (1923) and Martens (1932-3). As a result of his observation that lines of protoplasmic streaming, in staminal hairs of *Tradescantia*, are parallel to striations in the wall, Denham suggested that alignment of cellulose particles was brought about by the streaming of protoplasm, and it was not until Martens showed that the striations were part of a wall layer lying deep in the wall, and far removed from the protoplast by inner layers, that this idea was abandoned. Van Iterson suggests that the primary wall has transversely oriented cellulose chains (presumably again oriented by stress (van Iterson, 1937)), so that the wall can expand more readily in the longitudinal direction. This elongation is

supposed to change the direction of protoplasmic streaming until it lies longitudinally, when a new wall layer is deposited with cellulose chains lying in this new direction. If this new wall layer can expand, it will do so transversely, involving a return of the streaming to its original direction. The mechanism was originally introduced as an interpretation of the wall structure of *Valonia*, and has already been criticized (Preston & Astbury, 1937). It is difficult to visualize a change in streaming direction due to the relatively slow vacuolation, and in any case the idea ceases to be acceptable at all for a wall more than two or three layers thick. Whatever the mechanism of deposition may be, in cells like those of *Valonia*, it is certainly true that the cell seems to be capable of depositing in alternate layers sets of cellulose chains at a definite angle to each other. This is perhaps most clearly demonstrated for *Cladophora* (Preston & Astbury, 1939), but is also probably true in cells like the cotton hair, where the primary and secondary walls differ in chain direction (Anderson & Kerr, 1938).

At various times attempts have been made to deduce the structure of the primary wall from that of the secondary wall deposited upon it. Such attempts involve the general assumption that the structure of the secondary wall is to some extent governed by that of the primary wall, and the degree of success with which observed facts can be correlated leads one to suspect that this idea is not unreasonable. It was early suggested by Frey that the primary wall of the fusiform initials of conifer cambia are composed of micelles inclined at an angle to the vertical, giving the effect of a spiral. This idea has received support from observations of the change in wall structure of spring tracheids from one annual ring to another (Preston, 1934). It is a very striking fact that, as the cambial initial elongates, the micelles in the secondary layer of the radial walls, in the tracheids which it cuts off, become gradually more steeply inclined to the horizontal (as judged by the major extinction position, see p. 297). Again, the change takes place in no random fashion but strictly according to the equation

$$L = k \cot \theta,$$

where L = length of cambial initial (or tracheid), θ = angle of inclination of the micelles to the vertical, and k = constant. This equation, corresponding to the elongation of a spiral of constant girth, when considered with the fact that the cambial initials are themselves expanding at constant girth (Bailey, 1915, 1920; Preston, 1934) is clearly suggestive. At the very least it must be admitted that the simplest explanation is that both the cambial initial and the spring tracheid are wound with a *single* spiral. In this regard, it is not without significance that the thickened regions of the primary wall (Priestley & Tupper-Carey, 1922; Anderson & Kerr, 1938) are often spirally inclined, and that in conifer cambium the inclination is much what the above suggestion would indicate.¹ Consideration of the development of cambial initials from the shoot apex leads inevitably to the suggestion that these, too, are built up on a similar plan. Similar ideas have also been successfully

¹ Privately communicated by R. Greenhalgh as a result of some preliminary work commenced some years ago, the continuance of which has been unavoidably postponed.

used in consideration of the development of the vessel wall in ring-porous dicotyledons (Preston, 1939) and in the elongation of the oat coleoptile (Preston, 1938).

It seems feasible, then, that the primary wall is generally built up of micelles inclined to the horizontal, and that change in cell dimension can modify this inclination much as it has been observed to do in the parenchyma cells of the oat coleoptile. Whether such changes are due to passive extension of the wall or to active wall growth is not yet clear. This point has already been briefly discussed in various places (see e.g. Boysen Jensen, 1936; Preston, 1934, 1938). On the other hand, the protoplast itself may clearly play a part both in changing the orientation of cellulose micelles (from layer to layer in a wall) and in originating the specific orientation itself; in fact it seems probable that the old wall can orient the micelles in new wall layers only when acting either in conjunction with the protoplast, or sufficiently powerfully to overcome an opposing protoplasmic mechanism. A clear case of the orienting power of protoplasm is furnished by the tangential wall of conifer tracheids, the inclination of whose micelles, though different from that of the radial walls, remains the same over many annual rings (Preston, 1934).

While those cells whose walls are composed of more than one set of cellulose chains (*Valonia*, *Cladophora*, etc.) present a problem whose final solution is still far to seek, it seems probable that similar conceptions are applicable. Especially in the case of *Cladophora* is it possible to explain all the observed phenomena in terms of variation in cellulose chain direction with cell dimension, and of such a periodic modification in the orienting mechanism that the cellulose chains in a new layer make a constant angle with those of the last layer deposited. It seems highly probable that here the protoplasm plays a very active part in the orientation of the cellulose—indeed, it is not unreasonable to suggest that the alteration in the structure of the layers is brought about by a change in the configuration of the protoplasmic surface. The whole field of wall structure in the algae promises a study of the highest interest, and undoubtedly deserves more attention than it has hitherto received. There are already several cases which suggest that the structure of the protoplasmic surface and of the wall are not unrelated, and more complete structural analyses promise results of value in the application of wall structure to developmental problems.

Current suggestions as to the nature of the orienting mechanism in the protoplasmic surface are invariably speculative, as they must be in view of our present lack of knowledge of the finer details of protoplasmic structure. The conception of protoplasmic streaming as an orienting mechanism certainly has its attractions, but the evidence in its favour is hardly convincing. A more promising speculation centres on the structure of the protoplasmic surface itself. For many years the conception of the protein constituent as the structurally important feature of protoplasm has steadily been gaining ground, and the abundant evidence that protoplasm is fibrillar in nature (Seifriz, 1929, 1930, 1934, 1936) clearly points to an orientation of the protein molecules. Further, as Fischer originally postulated, proteins clearly consist of long chains of amino acid residues, much as cellulose consist of chains of glucose residues; the stereochemistry of the proteins has, in fact,

been worked out in some detail (Astbury & Woods, 1932, 1933; Astbury *et al.* 1935*b*; Astbury, 1937). The significance of these facts can hardly be overemphasized, since the protoplasmic surface itself is largely protein in nature, and in view of the abundant evidence of orientation at interfaces. The recent evidence put forward by Astbury & Bell (1938), that in thin surface films of proteins the molecular chains are preferentially oriented in the surface, is a notable contribution to this line of argument. In brief, it may well be that the orientation of cellulose chains in the wall is brought about by a similar orientation of protein chains in the protoplasmic surface.

Further progress in the application of wall studies to growth problems calls for close co-operation between the cytologist, anatomist, physicist and chemist. Only through a more detailed knowledge of the structure of protoplasm and of primary and secondary walls, together with a close study of their interrelationships, may the botanist hope to make full use of the tool which wall studies place in his hands.

VI. SUMMARY

The impetus which modern work on growth has given to wall studies promises to combine these into a branch of plant science of considerable importance to botanists in many fields. The present review represents an attempt to discuss, in relatively simple terms, the work which has led to present views on the structure of cellulose, the chief structural polysaccharide of the plant cell wall. To this end, the results of organic chemistry, X-ray analysis, and of polarization optics, are mentioned, and data from other sources are summarized where necessary.

Cellulose is shown to consist of long molecular chains, of which the links are β -glucose residues bound together, in the chain, by primary valences. The linkage is universally of the 1:4 type. The chains themselves are in turn bound together by secondary valences, or van der Waals forces, into ill-defined bundles which correspond to the classical micelle of Nägeli. Incrusting substances like pectin and lignin are certainly deposited in the intermicellar spaces, as against hemicelluloses like xylan, which are equally certainly intramolecular. The evidence for the existence of micellar aggregates, both in the wall itself and in the cytoplasm, is discussed in some detail.

In any one layer of the secondary walls in the majority of plant cells, the cellulose chains form a single spiral round the cell. With many fibres and tracheids this spiral retains the same sign, and approximately the same pitch, from layer to layer of any one wall. On the other hand, both the sign and the pitch of the spiral varies widely in some cell types. This has been demonstrated most clearly in the algae (e.g. *Valonia*, *Cladophora*, *Chaetomorpha*), where the cellulose chains of odd layers, say, point in exactly the same direction, but at a considerable angle (some 83° in *Valonia*) to the direction of those in the even layers.

In spite of the increasing prevalence of the conception that the cellulose chains in the primary wall lie transversely, it is quite clear that in those cases critically examined they form a spiral resembling that in the secondary wall. It would seem

that the development of a spiral in the secondary wall is not unconnected with its existence in the primary wall.

The various theories of wall growth which are based on a transverse orientation of the cellulose chains, in the primary wall, must clearly be abandoned. Such evidence as there is points to a change, during growth, of the inclination of an original spiral.

The nature, both of the cell wall and of the cytoplasm, is probably involved in wall deposition. Thus, a new layer deposited on a wall may be so influenced by the existing layers that the cellulose chains composing it lie parallel to those of the old wall. Yet the fundamental orienting mechanism must lie in the cytoplasm, for each wall originates as a new layer at cytokinesis. The evidence pointing to protoplasmic streaming as the mechanism involved is hardly convincing, and it seems not unreasonable to suggest that the configuration of the protein molecules at the cytoplasm-wall interface may be involved.

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AUXINS AND THE INHIBITION OF PLANT GROWTH

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(Received 29 September 1938)

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I. INTRODUCTION

IN recent years increasing attention has been paid to the role of hormones in plant development. It is beyond the scope of this review to outline all that has been done in the field, since the literature has been dealt with in a review by F. A. F. C. Went (1935¹), and more recently in two monographs (Boysen Jensen, 1936; Went & Thimann, 1937). In brief, it has been shown that growth is controlled by special substances, produced principally in apical parts such as buds, leaves and the tips of coleoptiles, and especially during periods of rapid development. These substances are transported to points distant from the place of formation, and the growth in these distant points is a function of the amount of hormone which reaches them, though, of course, nutritive (non-hormonal) factors also play a part. Growth may also be brought about in or near the zone of hormone production. In grown plants the substances are formed mainly in the light, but in seedlings formation also takes place in the dark.

The chemical nature of the hormones is rather well understood, and for convenience they have been termed auxins. Two auxins, auxentriolic acid or auxin *a* ($C_{18}H_{32}O_5$) and auxenolonic acid or auxin *b* ($C_{18}H_{30}O_4$) have been isolated from higher plant material, and one, indole-acetic acid ($C_{10}H_9O_2N$) from fungi, while a number of other compounds, not known to occur naturally, have the same or similar physiological effects. In addition to the auxins certain other substances act as growth hormones, though probably to a lesser extent. Among these is vitamin B₁ or aneurin, essential for root growth, and there is also some evidence that vitamin C, biotin and the animal sex hormones may act as plant growth substances (see Thimann & Bonner, 1938²).

¹ *Biological Reviews*.

² This covers also most of the literature appearing since the above-mentioned monographs.

It is well established that in stems, leaf-veins, petioles and coleoptiles the auxin always moves in a predominantly polar direction, i.e. from apex to base. This polarity has an internal, and not an external, cause, and is responsible for many morphological effects.

The various activities of the auxins are the following:

(1) they promote growth by cell enlargement in shoots of all kinds, the distribution of auxin in different parts of the shoot being the major single factor controlling growth;

(2) they are essential for the initiation of roots on stems and probably also for formation of branch roots on roots;

(3) they promote cell division in the cambium and in certain other tissues;

(4) they apparently promote guttation or bleeding from wounded surfaces.

On the other hand, the auxins also have growth-inhibiting action:

(5) they inhibit the development of lateral buds, causing them to remain dormant so long as they are supplied with auxin from other parts of the plant; and

(6) they inhibit the elongation of roots.

These two inhibiting properties stand in such sharp contrast to the general activity in promoting growth that it is proposed to consider them here in detail. The phenomena of inhibition comprise an important part of the physiology of plants which can best be understood by bringing them together in this way. There is also an important minor point, namely that there are cases in which auxins are found to inhibit growth of shoots. These will be discussed first.

II. THE INHIBITING EFFECT OF AUXINS ON SHOOTS

This inhibition occurs mainly with concentrations of auxin considerably higher than those which promote growth in the same material. Thus Czaja (1935) found that an auxin preparation from urine caused *Helianthus* hypocotyls to grow 127% as much as controls, while four times this concentration produced a growth only 19% of that of the controls. With *Avena* coleoptile sections immersed in auxin solutions, Bonner (1933) found that there was an optimum concentration above which elongation was reduced again. Scheer (1937) and Schneider (1938), applying auxin in agar, both found a reduction in effectiveness at high concentrations, though the growth was still higher than that of the controls. However, with immersed sections the higher concentrations give an actual inhibition. Using indole-acetic acid, Thimann & Schneider (1938) found that the elongation in 0.4 mg. per l. was 185% and in 10 mg. per l. 257% of the control sections in water, i.e. marked growth promotion was taking place, but in 40 mg. per l. the elongation was only 20%. The sections were probably damaged by the concentrated auxin.

The exact concentration at which growth is reduced below that of controls in water varies with the plant, the substance used as auxin, and also with the general sensitivity of the plants on the day of the test. On the whole, for aqueous solutions, it is about 50 mg. per l., or one part in 20,000, or 3×10^{-4} molal.

An interesting special case is provided by the petioles of the cotyledons of *Pisum*

(Dostál, 1936). If auxin in lanoline be applied to the cotyledons, the petiole elongation is promoted by low concentrations and inhibited by high (5 mg. indole-acetic acid per g. of paste). *Vicia* and other members of the tribe behave similarly but respond to different concentration ranges. The cotyledons themselves inhibit elongation of their petioles, but whether this is due to their producing a sufficiently high auxin concentration is not clear, since the radicle (which would not be expected to supply much auxin) also inhibits. Furthermore the plumule, which certainly does supply auxin—as shown by its vigorous inhibition of the bud in the cotyledonary axil—only very weakly inhibits the elongation of these petioles. Thus, although the auxin effect is clear enough, and provides a good example of inhibition, the natural correlations seem rather complex.

A corresponding phenomenon is observable in the streaming of protoplasm. In the epidermal cells of the *Avena* coleoptile, Thimann & Sweeney (1937) showed that low auxin concentrations, of the order of those which accelerate growth, increase the rate of streaming, several auxins having the same effect. Concentrations of indole-acetic acid above 10 mg. per l., on the other hand (Sweeney & Thimann, 1938), cause the streaming to slow down continuously. This inhibition is, at least in part, irreversible.

Similar effects are evident in propagation experiments. When cuttings are treated at the base with auxin, there is a concentration which is optimal for root formation, a small further increase giving a reduction in the number of roots, while a considerable increase leads to high mortality, often with rotting up to the level at which the cuttings were immersed in the solution. Roots may still be formed, however, above the rotted parts. Such damage effects may be produced in herbaceous cuttings at concentrations of 25 mg. per l., in woody cuttings usually at 100–200 mg. per l.

It is clear, then, that growth inhibition by these relatively strong auxin solutions is on the border-line of toxicity, and takes place near those concentrations which produce definite damage.

An inhibition of a slightly different nature may exist. Le Fanu (1936) found that if auxin, in lanoline paste, were applied at the basal end of an internode of *Pisum* it inhibited the elongation, while the same paste applied at the apical end promoted elongation of the internode. Her experiments were not convincing, as pointed out by Nagao (1937), but they have recently been confirmed by Snow (1938). A paste containing 0.5 mg. indole-acetic acid per g. had practically no effect on growth when applied from above the internode, but when applied from below it reduced the growth to about one-third that of the control. (The absence of any marked growth-promoting effect when applied from above is doubtless due to the presence in the tissue already of an optimal auxin supply, as was made clear by Thimann & Skoog, 1934.) When a concentration three times as high was used it inhibited growth if applied at either end, though more from below than from above. Pohl (1937) similarly found that auxin in lanoline, when applied low down on *Avena* coleoptiles, somewhat inhibited elongation of the part above it, but promoted elongation in the zones below. Similar observations with extremely high auxin

concentration (30 mg. per g. of paste) were made on *Phaseolus* by Mitchell & Martin (1937).

In another experiment Snow (1938) slit an entire pea plant in two from the base as high as the 5th node, leaving the halves joined above. If auxin in lanoline were now applied to one of the halves at the third internode, it not only inhibited elongation of the fourth internode above it, but also that of the corresponding fourth internode of the other half, which was only connected through the intact part above. Thus the inhibiting influence was exerted not only directly above the auxin, but could also travel first upward and then down on the other side. The explanation for this phenomenon will be discussed in Section V.

To sum up, there is an inhibition of growth due to very high auxin concentration, observable both in whole plants and in isolated sections. This is doubtless a direct effect. The response of stem tissues to auxins is thus represented by an optimum curve, of which the peak is reached, for indole-acetic acid and several other auxins, at about 10 mg. per l. There is also an inhibition which is perhaps of another type, observable on whole growing plants. This may be more of the nature of an indirect effect, and is exerted on the parts morphologically above the place of application. It is evident also that no reliable conclusions can be drawn from the indiscriminate application of auxin to plants, without careful control of concentration.

III. THE INHIBITION OF ROOTS

According to the Cholodny-Went theory of tropisms, the tropistic curvatures of shoots are due to an asymmetric distribution of auxin in the curving organ. In phototropism it is the shaded side which receives the more auxin,¹ in geotropism the lower side. The direct proof that this occurs was first given, for phototropism, by Went (1928), and for geotropism by Dolk (1930, 1936).

Now the geotropism of roots is towards gravity, that of shoots away from it, and correspondingly it was shown by Cholodny (1924, 1926, 1931) that the response of roots to sources of growth hormone is opposite to that of shoots. Thus decapitation of the *Avena* coleoptile decreases its growth rate, while decapitation of roots in many (but not all) cases accelerates their growth. Correspondingly also, the application to decapitated roots of their own root tips, or of coleoptile tips, retards their growth. Hence Cholodny (1926) pointed out that the opposite signs of the tropisms of roots and shoots fit in with the fact that they react in opposite ways to the growth hormones coming from their tips. As we should now say, the growth of shoots is accelerated by auxin, but that of roots is retarded.

While experiments with tips are of course not entirely convincing, proof that an extracted preparation of auxin inhibits root elongation was given by Nielsen (1930), with an extract of the culture medium of *Rhizopus sinuatus*, which he had found to be rich in auxin. This auxin was later shown (Thimann, 1935) to be indole-acetic acid. Navez (1933) and Boysen Jensen (1933) made similar experiments with extracts, but it was finally Kögl *et al.* (1934) who showed that pure auxin *a*, auxin *b* and indole-acetic acid exert this inhibiting effect, the former two compounds being some

¹ Phototropism is, however, complicated by auxin destruction and other factors.

five times as active as the latter. Meesters (1936) showed further that the inhibition is not due to the acidity of the solutions. Neutralizing the solutions does decrease their inhibiting power, nevertheless (Marmer, 1937), but this is because the undissociated auxin acids penetrate the tissues more readily than their salts (Albaum *et al.* 1937; Thimann & Schneider, 1938). With *Avena* roots the inhibition can be used as a simple method for auxin assay, since it is exerted at far lower concentrations than those of toxic metallic ions like mercury or silver (Lane, 1936). It is about as sensitive an assay method as the curvature of the *Avena* coleoptile, though of course it is open to many more objections.

The aerial roots of the tropical vine, *Cissus gongyloides*, behave in the same way as seedling roots. Auxin pastes made from orchid pollen (Laibach, 1933 *a*; Andreas, 1937) or from pure auxins (Zimmerman & Hitchcock, 1935; Andreas, 1937) cause positive curvature when applied to one side of the root, or inhibit elongation when applied symmetrically to the tip.

In contrast to the inhibition, extremely low concentrations of auxin cause a small but definite acceleration of root elongation. The evidence for this important point comes from many sides. Amlong (1936) decapitated *Vicia* roots and allowed them to stand for 3 hr. to deplete their internal auxin supply. If now 10^{-9} molal solution of indole-acetic acid were applied, the growth was accelerated; if it were applied one-sidedly, curvature was away from the side of application. Fiedler (1936) found that when *Zea* roots were grown in culture medium containing yeast extract, all concentrations of added auxin inhibited elongation, but when the yeast extract (which contained some auxin) was omitted, then 2×10^{-9} molal indole-acetic acid accelerated growth by some 30 %. With the same material Geiger-Huber & Burlet (1936) independently found that the optimum concentration of indole-acetic acid was about 3×10^{-11} molal, which caused an elongation 50 % greater than that of controls. Using the data of Thimann (1934) on the auxin content of *Avena* roots, as determined by chloroform extraction, these authors calculate that at this concentration the roots receive an amount of auxin about equal to that which they already contain. It follows that the amount of auxin normally in these roots is not far from the optimum for their growth, so that further addition could be expected to accelerate growth only slightly. Thimann (1936) found that if the shoots of *Pisum* seedlings were removed just above the cotyledon, and auxin in lanoline were applied to the stump, the growth of the roots was accelerated. In view of the conclusion of Gorter (1932) and Nagao (1936) that auxin is transported very little through the basal zones of the root, and is destroyed in them very fast, it is probable these pea roots received only very small amounts of auxin. In the same experiments the application of auxin to the coleoptile stump was not found to accelerate root growth in *Avena*, from which Thimann concluded that *Avena* roots contain already more auxin than pea roots, so that a small additional supply more readily inhibits their growth (cf. also Faber, 1936). In all these experiments the inhibition produced by auxin may be complete, but the maximum accelerations found are always small, so that it remains an open question as to how far auxin takes part in normal root elongation.

Another contrast to the inhibition is the subsequent behaviour of roots after the auxin has been removed. If *Avena* roots are inhibited by auxin for a day or so and then transferred to water their subsequent growth is hastened and eventually their growth exceeds that of controls (Thimann & Lane, 1938). The shoot growth may be accelerated also, as was observed earlier by Cholodny (1936). Also *Raphanus* seeds which have been soaked in concentrated indole-acetic acid solutions (175 mg. per l.) afterwards produce larger radishes than the controls (Amlong & Naundorf, 1937). The explanation of these "after-effects" of auxin treatment is not clear, and probably includes several factors. It may be that as auxin is destroyed in the root its concentration falls till it reaches the accelerating level. This is supported by Thimann & Lane's finding that the lower the auxin concentration used for inhibiting, the sooner does acceleration begin. On the other hand, the relatively high concentrations used increase greatly the number of roots on the seedling (Lane, 1936; Thimann & Lane, 1938), and this is probably responsible for the increase of vegetative growth. However, Pratt (1938) has found that there is also an after-effect on respiration. Wheat seedlings which had been soaked 18 hr. in indole-acetic acid gave 45 hr. later an increase in the respiration per unit dry weight. Since, however, their growth remained inhibited in all the concentrations used, the actual respiration per embryo was greatly decreased. In other words, the amount of respiration was not decreased proportionately to the growth, a fact which is interesting but hard to interpret.

Before it had been found that very low auxin concentrations may accelerate root growth, the inhibition was explained by Czaja (1935) in the following way: the supply of auxin to the root comes from two sources, namely the seed or shoot, and the root tip. Whether or no auxin is produced in the tip of isolated roots has been under dispute for some time, but there seems no doubt now that if adequately nourished the root tips continue at least to contain, if not to form, auxin (see discussion in Thimann & Bonner, 1938). According to Czaja, these two "streams" of auxin are the reason for the inhibition; a single "stream" promotes growth, but two "streams" inhibit it. Czaja's experiments, however, provided little convincing support for this view, and apart from the fact that the rather hydrodynamical concept of auxin "streams" is quite unjustified, it has been shown that even if auxin be applied to isolated roots only from the apical end, inhibition may still be produced (Thimann, 1936). Further, this effect is independent of the presence or absence of the tip. Hence root growth can be inhibited by auxin coming in only one direction. Thimann also showed that, in *Pisum*, root growth may be promoted by applying auxin to the base although the tip is still present, in which case growth is promoted although auxin comes in two directions. The development of lateral roots may take place when the growth of the main root is inhibited (as by decapitation or by treatment with concentrated auxin) or when it is promoted. It must be admitted that the whole problem of the control of root elongation, as also that of the relation between main and lateral roots, is little understood.

It might perhaps be suggested that the inhibition exerted by auxin is due to some effect on transport of nutritive substances down to the root, because the root is, after

all, dependent upon the shoot for its nutrition. However, in several of the studies mentioned above, isolated roots in water or culture medium showed normal auxin inhibition. Even if the isolated roots are provided with ample vitamin B₁, which is essential for their growth, they are still inhibited by auxin (Bonner, private communication).

There seems, therefore, no doubt that the inhibition of root elongation by auxin is a direct effect on the root tissues. It is not compensated by increase in thickness but comprises a decrease in total volume and weight. The response of roots to auxin gives an optimal curve which differs from the response of shoots only in that the peak of the curve for roots is at an exceedingly low concentration while the peak for shoots is close to the concentration which causes damage, i.e. it differs quantitatively but not qualitatively.

IV. THE INHIBITION OF BUDS

Probably as soon as trees were cultivated it must have been discovered that lateral buds do not develop so long as the branch or shoot is intact, but only after the apex of the shoot is removed. On many trees, wherever the shoot is cut, the most apical remaining bud will develop. In some trees and herbaceous plants a more basally situated bud will develop first. The capacity of these buds to develop is therefore always present, but they are inhibited by the presence of the terminal bud on the same shoot. The term "apical dominance" is often applied to this phenomenon. After the lateral buds have grown out, each takes on the role of terminal bud and inhibits the development of laterals on its own axis. By suitable decapitation so as to cause bud development at the right places the shape of a tree may thus be controlled. Pruning therefore is not only to remove unwanted growth but also to cause development of buds which would otherwise be inhibited, particularly of short shoots or fruiting spurs.

The concept of inhibition as a type of correlation in plants seems to have been first made clear by Errera (1905), who at the same time put forward the related idea of specific inhibiting substances. Sachs's earlier theories of organ-forming substances included a bud-forming substance, but did not extend to the realization that buds are subject to inhibition. Errera started from the observation that in *Picea excelsa* or other conifers, when the apical shoot is removed or damaged, one of the lateral branches nearest the apex begins to grow vertically. The presence of the apex therefore controls the geotropism of these laterals. However, in experiments on *Araucaria* (carried out together with Massart) he found, as did Vöchting (1904), that here decapitation is not followed by lifting of a lateral branch into the vertical position, but by development of a new bud which grows vertically. Before decapitation, then, this bud had been inhibited. Development of the new bud also takes place if the stem be ringed just below the terminal bud. Hence the "inhibitory excitation" is carried by the bark. This experiment also shows that the inhibition can hardly be due to withdrawal of water in the transpiration stream, since the terminal bud continues to grow (at least for a time) after ringing. Errera concludes that "le sommet envoie vers les rameaux des excitations inhibitoires qui les

empêchent soit de se développer (*Araucaria*) soit de se redresser (*Picea*)". He suggests that the action is due to "internal secretions" carrying inhibition downwards through the tree. A number of experiments of the same kind on *Pinus* have recently been described by Münch (1938).

This theory of inhibiting substances was not generally adopted. Goebel interpreted relations between buds in terms of nutrition. In *Juglans* he states that failure of the buds in the cotyledonary axils to develop is "due to all available food material being devoted to the development of one terminal bud" (1900). Pfeffer (1903), however, did not believe that insufficiency of food could be the cause, and points out that buds are often stored with food materials. Elsewhere he writes of "specific metabolic products" which might cause correlation. It is of interest that Loeb in 1917 attributed the inhibition of bud growth in *Bryophyllum* to the influence of inhibiting substances, but later (1923, 1924) ascribed it to "the laws of mass action". Thus in isolated internodes suspended in water or moist air, the amount of growth made by the axillary buds was found to be approximately proportional to the size of the stem section taken. If a portion several internodes long be used, only the most apical buds develop, whereas if the internodes are separated each supports growth of a pair of buds. The development of apical buds in the long section thus suffices to inhibit development of buds lower down. However, since the amount of growth which the buds put out is roughly proportional to the total amount of stem present in each case, Loeb considered that bud growth is limited by something distributed in the stem. If one pair of (apical) buds grows, they consume the available substance, preventing growth of other buds. But since Loeb's experiments in most cases lasted a month, during which nothing but water was given, the results are more probably due to the limitation of growth by nitrogen, potassium, or other essential nutrient. A series of equally naïve experiments on bud development from isolated *Bryophyllum* leaves, kept on wet filter-paper, is susceptible of the same explanation, and indeed, E. Reed has shown (1923) that if only the number of buds beginning growth is taken as criterion, there is no correlation at all with the amount of tissue.

The experiments of Reed & Halma (1919) provided clearer evidence in favour of an inhibiting substance, and were so interpreted by them. Notching of the stem of *Citrus medica* just above a lateral bud was found to cause its outgrowth. If a cutting was suspended horizontally, buds developed on the upper side only, as found also by Loeb (1917). On now rotating the cutting through 180° buds began to develop on the opposite side, while the first ones became inhibited. Thus the inhibiting influence travels morphologically downward, and also moves laterally under the influence of gravity. There are no indications that nutritive factors are concerned.

A variant of the ringing experiment of Errera and Massart was carried out by Harvey (1920) who killed a zone of the stem of *Phaseolus* by exposure to steam, when lateral buds developed below the dead zone, although the part above remained alive. In *Bryophyllum*, it is enough to chill a zone of the petiole to about 0° to cause outgrowth of buds on the leaf beyond the chilled zone (Child & Bellamy, 1920).

Thus the inhibition is carried through living tissues, and in most cases through the cortex.

That lateral buds may be inhibited not only by a terminal bud, but also by a leaf, was shown in Loeb's experiments, but in much more detail by Dostál (1926) for *Scrophularia nodosa*. In isolated internodes with one pair of leaves, he found that the leaf inhibits growth of the bud in its own axil, but a vigorously growing bud in the opposite axil exerts a much greater inhibition. Many other interesting experiments were described by Dostál, such as the influence of cuts in different places, but his interpretations were along the time-honoured lines of nutrition and water relations. The great inhibiting power of growing buds is largely to be ascribed to the very young leaves in them, which inhibit more strongly than mature leaves, as was shown later by Weiskopf (1927) and by Snow (1929).

The first definite evidence that bud inhibition is caused by a substance is due to Snow (1925), who showed that when a *Phaseolus* seedling was slit into halves which were bound together, the buds on one half could still be inhibited somewhat by the growing apex on the other half. The inhibiting influence may therefore cross a discontinuity. This experiment may be compared with Boysen Jensen's demonstration (1910, 1913) that the influence of the tip of the *Avena* coleoptile on the phototropic curvature of its base may also pass a discontinuity.

Final proof that inhibition is due to a special substance, as well as the identification of that substance with auxin, was given by Thimann & Skoog (1933, 1934). Starting with determinations of auxin in the plant, they found that in *Vicia faba* the terminal bud was the most active auxin-producing centre. Production of auxin by larger leaves was found to decrease rapidly with age, so that this high production in the bud is doubtless in large part due to the very young leaves. Production also decreases with age in *Nicotiana* (Avery, 1935) and in *Solidago* (Goodwin, 1937). Thimann & Skoog found that dormant axillary buds produce almost no auxin, but production begins as soon as they begin to develop. The auxin production thus parallels closely the inhibiting power of the various organs, and when it is further remembered that auxin transport resembles that of inhibition in being mainly from apex to base, and that auxin, like the inhibiting influence, may be diverted laterally by gravity, the parallel is seen to be complete. They completed the proof by removing the terminal bud and applying an auxin preparation (obtained from *Rhizopus* cultures and partly purified) in its place. The agar blocks containing auxin were renewed continually so as to provide a steady stream. The lateral buds were then inhibited as completely as those on intact controls. Aside from the inhibition of roots, this was the first demonstration that auxin has any effect other than that of promoting cell enlargement. In order to obtain complete inhibition, an amount of auxin several times larger than that which could be obtained from the terminal bud had to be used, the exact equal of that which diffused from the terminal bud giving only partial inhibition. This difference is doubtless due to inactivation of auxin at the cut surface as well as loss in the non-transporting tissues.

To discount the possibility of active impurities in the auxin preparation, the experiments were repeated with pure auxin *b* and indole-acetic acid, which gave

equally complete bud inhibition (Skoog & Thimann, 1934). A sample of pure auxin *a* which had become inactive on keeping was found inactive for bud inhibition, so that the inhibiting action evidently parallels the auxin activity.

At about the same time Laibach (1933) reported that the pollinia of certain orchids, which he had previously found to function as a source of auxin, produce partial inhibition of the development of the bud in the cotyledonary axil of *Vicia*. A more extensive study by Müller (1935) showed that such inhibition could be produced in a variety of plants, either by the pollinia or by a paste of urine and lanoline. Since the inhibitions were very incomplete, and were not carried out with pure or even partially purified auxin preparations, the deductions made by these workers as to the mechanism of inhibition (see below) are open to objection.

An interesting experiment was carried out by Uhrová (1934) on *Bryophyllum*, Loeb's old object. Here the leaf, which inhibits development of the bud in its own axil, was found to inhibit also if it were cut off and replaced. Or the leaf could be placed on agar and the agar applied to the cut surface of the petiole, causing inhibition. The loss of auxin by inactivation in this plant is therefore probably small.

In the ferns an interesting parallel to bud inhibition has been brought to light by Albaum (1938). The growing point of the heart-shaped prothallia of *Pteris longifolia* is situated at the centre of the apical indentation. If this is removed (and only if it is removed) adventitious outgrowths appear at the centre of the apex of the remaining piece, i.e. nearest the former growing point. When indole-acetic acid in lanoline is applied at this point the outgrowths are completely inhibited. If the same auxin paste is applied at the base the inhibition is partial. There is thus an apex-to-base polarity of the movement of auxin, and auxin was shown to be produced in the normal apex by diffusions and by extraction. The development of the outgrowths closely parallels the development of the most apical lateral bud in dicotyledons. In a later stage, removal of the growing sporophyte causes development of a new sporophyte at its base, and this latter may also be inhibited completely by auxin application. Thus, when the sporophyte begins to grow, the centre of growth and of inhibition (and presumably therefore of auxin production) shifts from the prothallium to the apex of the sporophyte.

There are two cases in which development of buds is apparently promoted by auxin treatment. One is that of *Lilium harrisii*, which, when decapitated and treated with auxin on the cut surface, forms buds in the axils of the upper leaves (Beal, 1937). Controls form no such buds, nor does the closely related species, *L. philippinense formosanum*. The buds later swell into bulbils. The other is the leaf of *Begonia rex*. Here Prévot (1938) finds that adventitious buds, which normally are produced only at the basal end of the isolated leaf, are produced at the apical end if auxin in lanoline is applied there. However, since Prévot had previously (1936, 1937) obtained the same result by dipping the apical part of the leaf in water or paraffin, or even by application of a certain bird manure to the growing plants, the effect does not seem to be very specific, and the action of auxin here may well be an indirect one. Where buds are formed from apical callus, as in *Populus*, Fischnich (1938) has made it clear that their appearance is inhibited by auxin treatment, just

as is the development of lateral buds. In any event such effects on bud *formation* are not to be confused with the action of auxin on bud *development*.

The remainder of the more recent literature will be discussed in Section V.

Bud inhibition and general morphology. Lateral bud inhibition is, of course, important in determining the habitus and general appearance of a plant. The difference between sympodial and monopodial growth is presumably the difference between prolonged auxin formation by the terminal bud, causing inhibition of laterals, and an auxin production which is only transient and therefore followed by development of the most apical lateral. Some of these relations were studied by Mogk (1914) but they have not yet been reinterpreted on a hormonal basis. It is, however, of interest that Zimmermann (1936) and Söding (1937) both find that auxin production by the terminal bud of tree twigs, and correspondingly cambial development below it, falls off after a few weeks (cf. also Avery *et al.* 1937). It would be interesting to know if there is correlation between this and lateral bud development. The control of the geotropism of lateral branches by the terminal bud (cf. Errera above) has not yet been explained. In propagation experiments it has been found that when lateral branches of *Picea* are rooted they retain their plagiotropic growth at least for a year, and this appears to be well recognized by horticulturists, so that whatever influence has been exerted upon the laterals is not readily reversible (Thimann & Delisle, 1939).

In herbaceous plants it is common for lateral buds to develop towards the end of the season, and correspondingly Thimann & Skoog found that in *Vicia* the amount of auxin diffusing from the terminal bud decreases with increasing size of the plant, so that at 37 cm. height no auxin could be obtained at all. The bushy habit of many dwarf plants probably has the same explanation. In a dwarf *Zea mays* (var. *nana*) van Overbeek (1935) found an increased rate of auxin destruction and therefore considerable reduction in the amount of auxin present. Such dwarfs produce numerous tillers. Similarly, Delisle (1937) found that in *Aster multiflorus*, in which many laterals develop, the auxin production by the terminal bud is less than in the related *A. novae-Angliae*, which is almost unbranched; the hybrid has intermediate auxin production and an intermediate extent of branching. Application of pure auxin inhibits lateral bud development in these plants as would be expected.

The experiments of Goodwin (1937) indicate that growing leaves may inhibit not only buds but also other leaves. When *Solidago* is in the rosette stage, removal of a growing leaf promotes development of the next in order, but application of auxin to the petiole has the opposite effect. Correspondingly the inhibition exerted by one leaf upon the next ceases at about the time its auxin production decreases.

Another important relation is that between auxin inhibition and "dormancy". Mogk (1914) pointed out that laterals which have been inhibited for most of the season make poor growth when the inhibiting influence is finally removed. It is well known also that if the inhibition lasts the whole season then the buds will not develop at all the following year unless a period of cold has intervened. Thus the buds on apple trees kept in the greenhouse over winter remain dormant the following spring. Molisch (1909) was able to stimulate such buds into activity by

treatment with hot water. Bennett & Skoog (1938) have now shown that this dormancy is in some cases released by injecting low concentrations of auxin, but the most effective treatment was with yeast extract and preparations made therefrom. The nature of the active substance is not known.¹ Although auxin inhibition is usually reversible (cf. Thimann & Skoog, 1934) it may be, at least in some cases, that if it is prolonged for many months the buds slowly lose their ability to grow, and even to respond to applied auxin, and some further change must now be brought about before growth can begin.

Dormancy does not, of course, result only from prolonged inhibition. In the *Hydrocharitaceae* special buds are formed in the autumn, and these, although they become detached from the plant, remain dormant till the following spring. Apparently this dormancy is due to lack of auxin. Vegis (1937) has shown that although their development can be induced by hot water treatment or by salt solution, more rapid development is caused by immersion in indole-acetic acid solutions of from 0.4 to 50 mg. per l. Vegis infers that the other effective methods act indirectly by setting free auxin. Treatment with acid buffer is also effective and this is ascribed to the well-known setting free of the active auxin acid from its salts (or other bound form) by strong acid (see Went & Thimann, 1937, Chap. 8).

Interactions of buds in tubers. The relations between buds in tubers are in some respects similar to those in stems. Appleman (1918) pointed out that in the intact potato only the apical bud usually develops, but if the buds are separated by slicing, or even by making furrows between them, all of them develop. Similarly, in *Scrophularia nodosa* tubers, Havránek (1931) showed that the influence of one bud upon another is carried by the cambium. According to Dostál (1936a), the lateral buds on these tubers are exposed to two inhibitions, one from the body of the tuber itself and one from the growing apical bud. If the latter is removed, application of indole-acetic acid in lanoline will inhibit the growth of the next lateral bud. Also if the tuber is sliced into flat disks, each containing two buds, application of auxin to the pith on one side will inhibit the bud on that side only, but application to the cortex will inhibit the buds on both sides. Hence the inhibiting influence of the auxin is carried, at least to some extent, around the tuber in the cortex. The main movement of auxin is from apex to base, however, as shown by root-formation, on the base, only below the point of application of the auxin. This suggests that in these tubers buds are inhibited by lower auxin concentrations than are needed for root formation. Other explanations are, however, possible.

Buds on tubers can also be inhibited by soaking in very concentrated auxin solutions (Guthrie, 1938). Treatment of potato pieces with 1000 mg. per l. of indole-acetic or naphthalene-acetic acids or their salts delayed sprouting for at least 2 weeks, although the tubers were not entirely killed because they formed roots very vigorously. Such delay in sprouting can, of course, also be produced by ethylene (Elmer, 1932; Huelin, 1933). It is interesting that the auxin-inhibited buds resemble those which are naturally dormant in that development is hastened

¹ Boysen Jensen (1936) also mentions that dormant winter buds on several shrubs could not be induced to develop by treatment with an auxin solution.

by ethylene chlorhydrin (Guthrie, 1938*a*), which was previously used extensively by Denny (1926) and others to hasten germination of dormant tubers of several plants.

V. THE MECHANISM OF INHIBITION

It is clear from the foregoing that, whatever be the mechanism, inhibition of a bud by a leaf or by another bud is due to the auxin produced by the inhibiting organ. Yet, as shown at the outset, auxin applied to the stem promotes growth unless its concentration is very high, or unless maximal growth response is already taking place. Hence the problem arises: how can auxin both promote and inhibit growth? The difference is not entirely that between the stem and the buds, because it was shown in Section I that there are occasions when stem growth is also inhibited. Typical natural inhibitions often involve shoots rather than buds, as was first made clear by Hallbauer (1909) and Mogk (1914).

Thus Hallbauer found that when the growth of the terminal bud of *Phaseolus* is arrested by encasing it in plaster of Paris the buds in the cotyledonary axils develop, but when the plaster is removed these axillary shoots are soon inhibited by the now growing terminal bud. In Dostál's experiments (1926) the two buds in opposite axils of *Scrophularia* both develop slightly, but if one is favoured by removing the leaf in whose axil it stands the other one soon becomes inhibited. Similarly, Snow (1931) showed that if the main shoot of *Vicia* or *Pisum* be removed so that two axillaries develop to approximately equal shoots ("two-shoot plants"), these inhibit one another. Again, the measurements of Thimann & Skoog (1934) with *Vicia* show that when two buds are allowed to grow the shorter one grows only for a time and is then checked by the longer one. If the latter is removed growth of the shorter one increases again. Such shoots are thus in a state of balance, and any reduction in the auxin content of one gives an advantage to the other.

Another important point is that apparently any substance which acts as an auxin also causes bud inhibition. The approximately equal activity of auxin *b* and indole-acetic acid was mentioned above. Thimann (1935) showed that indene-acetic and benzofurane-2-acetic acids, both of which are weak auxins, inhibit bud development in *Pisum*. They are apparently not transported as readily as indole-acetic acid or the natural auxin in the plant, since the inhibition they produce is not strong unless they are applied within a few cm. above the lateral bud. Indole-propionic and naphthalene-acetic acids also inhibit, at least in high concentrations (Hitchcock, 1935).

Of the various theories which have been put forward to explain the facts of inhibition, there are nine which may be considered as distinct. Some of these have been discussed in detail by Snow (1937) and by Ferman (1938). The first two theories involve "direct action" of the auxin, numbers 3, 4, and 5 involve what may be called "diversion", and numbers 8 and 9 involve "indirect action".

(1) *Prevention of auxin formation by applied auxin*

Thimann & Skoog (1934) found that after decapitation, when the lateral buds begin to grow, considerable amounts of auxin become obtainable from them by

diffusion. They therefore postulated that the auxin which reaches the laterals (either from the terminal bud or from artificial application to the stem) has the effect of preventing the formation of auxin in these buds. Since the bud auxin is formed from a precursor (see Went & Thimann, 1937, pp. 64 and 213; Skoog, 1937) this would mean that the applied auxin prevents transformation of precursor into auxin. The fact that even within 24 hours after decapitation (van Overbeek, 1938) the amount of auxin begins to increase in the laterals, also points clearly to a suppression of auxin *formation* in the inhibited buds. Also Le Fanu (1936) found that in inhibited shoots of a "two-shoot plant" less auxin than normal was being produced, and this is confirmed and extended by Ferman's observations on *Lupinus* (1938). Since enough auxin must reach the buds to prevent their forming auxin themselves, but not enough for them to grow, the concentration of auxin necessary to suppress auxin formation should be, on this view, quite low. Thimann & Skoog supported this by reference to the experiments of Söding (1925), which showed that application of the tip to previously decapitated coleoptiles suppressed the new formation of auxin ("physiological regeneration") in the stump.¹ In this respect their conclusions have been somewhat misinterpreted by Snow (1937) and Le Fanu (1936).

(2) *Inhibition by too high an auxin concentration*

A modification of the above view was suggested by Thimann (1937). When auxin in lanoline was applied directly on to the lateral buds, he found that inhibition was about as complete as when it was applied to the stem. Even concentrations as low as 0.04 mg. per g. of lanoline reduced the bud length to about one-half the normal. Plch (1936) had obtained the same result when a paste containing 2 mg. per g. was applied to the cotyledonary axil, and Dostál (1937) could even inhibit the terminal bud by direct application in this way.²

The behaviour of fasciated buds parallels that of normal buds. According to Schoute (1936) fasciation is due to lateral widening of the growing point. In *Phaseolus multiflorus* the buds in the cotyledonary axil have a marked tendency to become fasciated, and Tutschová (1937) has been able greatly to decrease the percentage of fasciation by applying auxin either to the cotyledons, to the roots or to the bud itself. Correspondingly the application of eosin, or irradiation with ultra-violet light (both of which would destroy auxin in the plant) increased the percentage of fasciation. Here, then, the lateral extension of the growing point is directly inhibited by auxin.

The developmental processes of young buds are thus inhibited by auxin concentrations which would not inhibit stem growth. On the other hand, there is no reason to suppose that the enlargement of cells in buds differs in principle from the

¹ However, Söding's findings might, on the basis of present knowledge, be ascribed to loss of the auxin precursor by diffusion into the applied tip.

² On the other hand, the actual leaf primordia may be increased in size when auxin is applied direct to them (Snow & Snow, 1937) or they may be caused to develop abnormally when auxin is applied externally to the bud (Laibach & Mai, 1936; Tutschová, 1937). Since bud growth as usually determined is essentially elongation of a young stem, the influence of auxin on leaf growth need not be considered here.

enlargement of cells elsewhere, and presumably, therefore, it requires auxin as catalyst, though perhaps only in minutest amounts. When it is further considered that, in Thimann's experiments (1937), the lowest auxin concentrations actually increased the weight of the buds somewhat, it becomes clear that the response of buds to auxin is given by an optimum curve, like those for stems and roots discussed in Sections I and II. The peak of this curve, however, lies in very low auxin concentrations, perhaps even approaching the position of the peak for roots. Since the concentrations are low it is improbable that inhibition of buds lies on the borderline of toxicity as it does for stems. The amount of auxin present in the stem of intact plants, causing elongation there, would be high enough to inhibit bud growth.

The objection to this as a general theory of inhibition is that it would not explain inhibition of whole shoots, as in "two-shoot plants" (Snow, 1937). However, it is not yet certain that the latter is merely the same phenomenon as inhibition of buds; it may involve additional factors. In any event the experimental fact that young buds are inhibited by the *direct* action of relatively low concentrations of auxin must always be considered in forming a complete picture of inhibition.

(3) *Diversion of food supply*

As mentioned above, an early view was that any part which is already growing uses up the available nutrients and thus diverts them from other parts, which are consequently inhibited (Goebel, 1908; Loeb, 1915, 1923). Loeb's experiments are open to the very serious objection already raised (p. 321). Further, the theory has been applied with some elasticity, for Loeb also argued (1922) that the reason why a piece of attached stem reduces the amount of bud growth is because "materials which could have been utilized for the formation of new shoots and roots in the leaf now goes into the stem". Here no claim was made that the stem was growing.

To imitate the diversion effect, Moreland (1934) applied suction to the apex of decapitated *Phaseolus* plants. The solution drawn through contained both sugar and nitrate, by qualitative tests. Development of the lateral buds was markedly inhibited, but the significance of this for normal bud inhibition is very doubtful. It must be remembered that in ringing experiments lateral buds may develop even while the terminal shoot remains active.

Bud development may, of course, be inhibited by starvation, but fully etiolated buds on isolated sections of pea stems can grow at least for a time, so that there is no reason to believe that food relations are important for the first days of growth. Also in the case of inhibition by simple application of auxin, the point to which the auxin is applied may show no growth at all (Skoog & Thimann, 1934; Thimann, 1937) yet inhibition is complete. This and the facts (a) that some inhibition is exerted by mature leaves which are not growing, and (b) that the buds in the cotyledonary axils of legumes are inhibited by the cotyledons themselves, which are the main store of food materials (Plch, 1936; Dostál, 1937), make this theory unacceptable.

(4) *Diversion of other growth factors*

An important modification of Loeb's view was that of Went (1936, 1939). In experiments on the removal of cotyledons and roots from pea seedlings, evidence was adduced that the elongation of buds is dependent on some factor which comes upwards from the roots (see also Went, 1938). This factor is considered to be a special stem-forming substance in the sense of Sachs. Comparable evidence that bud development in rhizomes is controlled by some factor which moves towards the apex has been given by Schwanitz (1935). Went's suggestion is that auxin controls the movement of this substance, and that the terminal bud, or any auxin-producing centre, will attract the bud-growth substance toward itself and thus divert it from other buds. As soon as the laterals begin to form auxin, they will begin to attract some of this substance to themselves and therefore grow.

The principal objection to this view is furnished by direct application of auxin, not to the stem, but to the lateral buds themselves (Thimann, 1937; see also Plch, 1936). Here, since the plants were also decapitated, the special factor should move to the laterals with increased intensity. Nevertheless the buds were inhibited about as strongly as if the auxin had been applied to the stem, and (if the auxin concentration were not too low) there was an inhibition of weight increase as well as of elongation. Further, on isolated pieces of stem the buds will grow out for a while, i.e. some of this factor must be stored in them or in the stem, so that the influence of the roots is necessary only for *prolonged* growth. Another difficulty arises from the experiments of Plch (1936) and Dostál (1937) on the inhibition of buds in the cotyledonary axils, an effect which can be either intensified or balanced by the proper application of indole-acetic acid or various natural sources of auxins. If one cotyledon be removed the bud on that side develops, and even if the root is incised or partly removed on that side the result is not affected, so that the development of that bud is certainly not directly dependent on anything coming from the roots.

Snow (1937) describes another experiment which cannot be readily explained by any of the "diversion" theories. A young *Vicia* plant was slit in two up through the roots, between the cotyledons, nearly to the first node. It was then decapitated and the buds in the cotyledonary axils allowed to grow out into shoots. The shoots, each with a terminal and lateral buds, are thus connected together only by the zigzag path of the slit main stem, yet each has direct access to one cotyledon and half the original root system. Nevertheless, when one of these shoots was decapitated, the lateral buds on it were shown to be still subject to inhibition from the terminal bud of the other shoot. It is certainly difficult to believe that any material coming from the cotyledon and roots could be diverted along the zigzag path to the growing shoot without first reaching the buds which are inhibited.

Snow also claims that this experiment cannot be explained by direct access of auxin to the inhibited bud, because the auxin from the growing shoot could only reach the bud by passing first down the shoot, then upwards against the normal polarity of its transport, in the slit main stem, down again and up in the second

shoot. However, transpiration was taking place, and Skoog (1938) has shown that upward transport of applied auxin in physiological concentrations may occur to a considerable extent in the transpiration stream. It is thus not impossible for auxin to have travelled in the direction specified. On the other hand the experiment mentioned under theory 9 shows that inhibition may travel upwards even where the influence of transpiration is excluded. In this connexion it is noteworthy that when auxin is applied to the base of the stem in cuttings, extensive bud inhibition is often caused, as may easily be observed in *Populus*, *Salix*, etc.

All these experiments make diversion of any factor coming from roots or cotyledons improbable as the *primary* cause of inhibition (see also Addendum).

(5) *Diversion of the auxin precursor*

In an important series of experiments which confirm for *Lupinus albus* many of the facts found by Thimann & Skoog for *Vicia faba*, Ferman (1938) made direct extractions of auxin from young shoots inhibited either by the terminal bud or by applied auxin, and also from lateral buds, normal and inhibited. Although many of the differences are small, his figures consistently show that the inhibited parts contain less auxin than the uninhibited. He concludes that inhibition is caused by lack of auxin formation (cf. theory no. 1). Now, as mentioned above, auxin is formed from a precursor which appears to be transported towards the apex and stored or deposited in buds. Ferman suggests that this precursor is attracted to the organ which is producing auxin and there is converted to still more auxin. The non-auxin-forming laterals receive no precursor. The process of auxin formation thus in some way attracts the precursor. Improving upon other diversion theories, he does suggest a mechanism for the attraction, ascribing it to the action of auxin in accelerating protoplasmic streaming (Thimann & Sweeney, 1937) which would thus promote transport in and through tissues receiving auxin.

This theory suffers from the disadvantages of the other diversion theories. (a) Direct application of auxin to the lateral buds should attract precursor to them and cause them to develop. The corresponding argument holds for the outgrowths on fern prothallia. (b) Even apart from attraction of precursor, if the buds were failing to develop from lack of auxin, the applied auxin should help their development (cf. theory no. 2). (c) When auxin is applied to the stem after decapitation, it should cause precursor to accumulate there and, if the point of application is close above a bud, growth of this bud should be promoted. This also does not occur (Thimann, 1937), although it is apparently true that inhibition is somewhat less marked when the auxin is applied near the bud (Went, 1939).

On this account Ferman considers that inhibition by applied auxin has another mechanism from that exerted by the terminal bud. This assumption of two different mechanisms seems highly undesirable and is not warranted by the facts, which all point the other way. The second mechanism postulated for the case of auxin inhibition resembles that of van Overbeek below.

(6) *Auxin clogs the channels of transport*

By direct extraction of lateral buds within 24 hr. after the inhibiting influence (terminal bud or applied auxin) had been removed, van Overbeek (1938) showed that in *Pisum* perhaps the first demonstrable change in the lateral buds is increase in the amount of auxin in them. Again the conclusion is that it is auxin formation which has been prevented. Van Overbeek suggests that the auxin supply clogs up the conducting tissues, thus preventing nutrients, etc., from reaching the buds. Since the actual amount of auxin is small it is supposed to be adsorbed on to the walls of the conducting cells, and since there is some evidence that transported substances move along the walls (cf. Schumacher's experiments on transport of fluorescein and aesculin, 1936, 1937) this would hamper general transport. To get over the difficulty that the growing bud ought thus to inhibit the transport of matter to itself it is pointed out that the vessels serving it are larger than those serving the laterals and hence are not so easily clogged.

There are several difficulties in this theory, particularly in the application to the inhibition of developed shoots. It is a remarkable fact that these last two theories claim to arrive at the same result, namely bud inhibition, by making essentially opposite assumptions; in the one the auxin promotes transport, in the other it inhibits it.

(7) *The two-stream theory*

According to Czaja (1935) inhibition only occurs when two streams of auxin are moving in opposite directions, while a single stream promotes growth. This view, in its application to root growth, was discussed in Section III, and there are no additional facts which make it any more acceptable for bud inhibition.

(8) *Inhibition results from an increased growth elsewhere*

Laibach (1933) and Müller (1935) considered that inhibition is a secondary phenomenon resulting from the fact that auxin increases the growth of some other part of the plant. In inhibition by the terminal bud the apex of the plant continues to grow. When high auxin concentrations are applied the bud inhibition is accompanied by swelling of the stems. In both cases this increased growth uses up material which would otherwise go into the lateral buds. The pollinia used in their experiments did cause extensive stem swelling. This view has something in common with the "diversion of food" theory, although Laibach did not specifically state that material was used in the growth, but only that it was the growth which in some way caused the inhibition. The best objection is that it is not necessary for any growth to occur in order that buds should be inhibited. Thus Skoog & Thimann (1934) showed that if the right auxin concentration were used, no elongation and no visible swelling resulted, yet inhibition of buds was complete. In more detail Thimann (1937) has shown that although the dry weight of the developing buds was greatly decreased by auxin applied to the stem, yet the dry weight of the stem was increased relatively little and in some cases not at all. There is therefore a real decrease in total dry weight, in other words, a true uncompensated inhibition.

(9) *Auxin sets free a special inhibiting influence*

Since there is no compensating growth, Snow (1937, 1938) has modified the theory of Laibach and Müller in the following way. The auxin brings about some process, "which according to circumstances may or may not be followed by actual growth", and it is this process which gives rise to the inhibiting influence. The important point is that the inhibiting "influence", whether it be a substance or not, can move in both morphological directions in the plant. Thus to regions below the point of application of the auxin both auxin and inhibitor move, and the effect of the auxin overcomes the inhibiting effect. To regions involving morphologically upward movement the auxin cannot penetrate to any extent because of the apex-to-base polarity of its transport, but the inhibitor can. The theory is supported by numerous experiments in which inhibition is produced at points which auxin could only reach by travelling morphologically upward. Thus in a "two-shoot plant" one shoot was decapitated and the cut end dipped in water, the roots being removed and no other water supplied. Water had thus to be taken up through the cut end. The lateral buds on the decapitated shoot were still inhibited by the other (growing) shoot, although the transpiration stream was travelling in the opposite direction to that of the inhibiting influence.

The slit plant experiment, described under theory no. 4, supports the same view. Further, defoliated side-shoots which are inhibited by another shoot show no cambial development (Snow, 1932), although, as Snow himself has shown (1935) auxin even in low concentrations stimulates the cambium. Thus Snow concludes that inhibition can travel where auxin cannot. Bearing in mind Skoog's transport experiments (1938), and the fact that the inhibitions here studied were always exerted by growing shoots and not simply by applied auxin, it is perhaps safer to say that inhibition certainly travels where auxin would not be expected to travel.

On the other hand it remains true that where two buds develop, it is usually the upper which inhibits the lower, so that inhibition travels better in the direction in which auxin can also travel. Thus in the experiments of Skoog *et al.* (1938), auxin applied to the stem of *Pisum* above the two lower buds inhibited them both; applied between the two buds it inhibited only the lower, and applied below both of them it produced no inhibition. Also in a number of experiments of the author (unpublished) auxin was found to inhibit much more strongly the development of a bud below it than of one above it.

The findings of Albaum (1938) on the fern prothallium are also not readily explained on this basis, since in this object auxin and the "inhibiting influence" have to move in the same direction, there being no intermediate region of opposite polarity. In this case all the observations point to auxin itself as the inhibitor. The simplicity of structure of the object, and the fact that the outgrowths never appear unless the auxin supply is reduced, strongly favour one of the first two theories. On the other hand the outgrowths are adventitious and, like adventitious buds, might perhaps not be strictly comparable with development of preformed buds.

Another difficulty about the theory is that it does not explain one of the best

established facts of inhibition, namely that there is a decrease in the amount of auxin in the inhibited part. The inhibitor might, of course, be envisaged as not actually inhibiting growth but inhibiting auxin formation, but this would make the theory almost indistinguishable from no. 1. The best support for this view of Snow would be, of course, the isolation of such an inhibitor and the proof that it is actually produced under the influence of auxin. Haagen Smit (private communication) has obtained from radishes a growth-inhibitor for *Avena* which contains auxin combined with some other unknown molecule; on hydrolysis it yields auxin. This discovery may well have an important bearing on bud inhibition, because such a compound can presumably only be formed from auxin itself. It is not yet known, however, how general its occurrence and inhibiting effect may be. For the present this theory must be regarded as one which remains open.

To sum up, it is evident that the inhibition of bud development remains an obscure process. All the theories are open to some objection, though some are obviously weaker than others, and more facts are needed before a decision can be made. In the writer's view, too little attention has been paid, up to now, to the rate and intensity of auxin destruction and inactivation in inhibited parts. A number of facts point to the importance of auxin inactivation as a factor in controlling growth. These include inactivation by light in phototropism, by X-rays in producing the growth inhibition which follows exposure to these rays, and by oxidizing enzymes in the dwarf corn. If inhibition were due to excessive auxin inactivation this would explain why so little auxin is found in inhibited parts, and also, possibly, why the direct application of auxin to inhibited buds does not promote their growth. The change from inhibited to non-inhibited state would then be due to a decrease in auxin-destroying tendency, and *vice versa*. The rate of auxin destruction would have to be controlled by auxin itself, but this need not be surprising in view of the numerous cases among micro-organisms in which formation of a certain enzyme is dependent upon the presence of its substrate (cf. Karström, 1930; Stephenson & Stickland, 1933; Stephenson & Yudkin, 1936). In brief, in tissues which are inhibited auxin would be destroyed as fast as it is formed (in buds) or supplied (in internodes). As soon as the auxin supply is temporarily cut off, the auxin-destroying system would fall off in intensity. Such an alteration in destruction rate might necessitate an important change in the metabolism of the bud or shoot. That there is some connexion with general metabolism is supported by the recent finding of Skoog in the writer's laboratory (unpublished data) that if *Pisum* buds are treated with vitamin B₁ at the same time as with auxin their inhibition is greatly reduced. Vitamin B₁ is known to play an important part in carbohydrate oxidations. The fact that the breaking of dormancy appears to involve metabolic changes may also be considered in this connexion. It is possible that the growth of isolated buds in culture medium would shed light on the problems of inhibition. As has been remarked before, however, since we are not yet able to explain the way in which growth is produced by auxin, it is perhaps premature to expect to explain the inhibition of growth.

VI. SUMMARY

Inhibition of growth is widespread in the plant kingdom. Just as the promotion of growth is controlled by the growth hormones, comprising principally the group known as the auxins, so also inhibitions are, in many cases at least, due to the action of auxins.

The application of very high concentrations of auxin inhibits the growth of shoots directly. Such concentrations retard the rate of protoplasmic streaming and are close to the range at which these substances are definitely toxic. Another effect which results from very high concentrations of auxins, and is perhaps of a more indirect nature, is the inhibition of the growth of parts morphologically above the point of the auxin application. Whether or not these phenomena of very high auxin concentration have any bearing upon normal growth inhibitions is still not clear. They may play a part in pathological inhibition.

The inhibiting effect of the root tip upon the growth of the root may be readily imitated by application of very low concentrations of auxin, probably of the order of those present in the tip. Hence this inhibition, where it occurs, is due to the auxin coming from the root tip. A somewhat lower range of auxin concentrations accelerates root growth. These effects are observable on isolated roots. The response of roots to auxin can thus be represented by an optimum curve with the peak at very low concentration. The growth of roots which have been inhibited may become accelerated when the auxin is removed; this may lead to a definite acceleration of shoot growth.

The inhibition of the development of lateral buds by the terminal bud of growing shoots can also be quantitatively imitated by the application of auxin in concentrations not much greater than those which are produced by the terminal bud. Hence this inhibition, the release of which comprises the basis of pruning, is due also to auxin, and in fact auxin is produced in rather large amount in young terminal buds of the majority of plants. The inhibition of buds by the leaves in whose axil they stand is similarly caused.

Not only buds, but also young developing shoots may be inhibited by another shoot or by auxin in suitable (physiological) concentration. The adventitious outgrowths on fern prothallia are subject to a similar auxin inhibition. In tubers the inhibiting action of one bud upon another is, at least in part, also an auxin effect.

Differences in bud inhibition between sympodially and monopodially growing trees, between normal and dwarf forms, or between related species of different growth habit, may be, and in some instances have been satisfactorily explained by, differences in the rates of auxin production or consumption. This concept unifies a scattered and diverse group of observations on plant behaviour.

However, the paradox that auxin, which typically promotes growth by cell enlargement in shoots, should inhibit growth of buds and of roots, has not been satisfactorily explained. The divergent views on bud inhibition have engendered nine theories. The principal point at issue is whether the inhibition is due to the auxin itself or to some effect of auxin on the production or movement of other

substances. None of these theories is entirely adequate to explain both the inhibition of buds and that of young growing shoots, though it is still possible that one of four may be established by further study. It is pointed out that the inactivation of auxin in inhibited parts may play an important role which has not yet been considered.

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ADDENDUM

Certain recent developments in connexion with bud inhibition call for comment. In the experiments of Went (1939) the growth of buds was accelerated when the stems had been first treated with auxin and later severed from the plant. The accelerations are ascribed to a mobilisation of bud-growth factors. However, it is noteworthy that the effects reported were obtained with indole-acetic acid concentrations too low to produce any marked bud inhibition under the same conditions. Concentrations high enough to produce undoubted inhibition exerted only inhibiting effects even when applied in this way. Thus the accelerations could equally well be due to an optimum auxin curve as in theory no. 2.

That auxin is responsible (directly or indirectly) for some kinds of mobilisation, however, is shown by the experiments of N. W. Stuart (*Bot. Gaz.* **100**, 1938, 298) in which carbohydrates and nitrogen were found to be moved towards the point of application of auxin. Nevertheless it is improbable that these play any major part in bud inhibition, since it has now been shown (Skoog, 1939, in press) that isolated buds, growing in nutrient solution, are inhibited by all but extremely low concentrations of auxin. Such inhibited buds grow freely upon removal of the auxin. This makes it clear that inhibition is exerted directly upon the bud, and that, irrespective of any evidence for the diversion of growth factors, such diversion can hardly be the main cause of normal bud inhibition.

EXPERIMENTAL STUDIES ON GENETICS OF FREE-LIVING POPULATIONS OF *DROSOPHILA*

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(Received 20 December 1938)

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I. INTRODUCTION

PROBLEMS relating to the genetic structure of free-living populations are not new. They were foremost in Darwin's mind, and sustained interest toward them has stimulated, if not directly caused, the rapid development of genetics at the beginning of the present century. Yet, strangely enough, genetics drifted farther and farther away from these problems, relegating them to the tender mercies of systematists, eugenicists, and even to the so-called racial theorists. The solution of this paradox is rather simple: as far as genetics was concerned the observational and speculative methods of the past seemed to have outlived their usefulness, and more exact methods were yet to be evolved. More recently the situation has begun to change again. The general acceptance of the theory of the gene, together with the availability of precise techniques of laboratory experiments, have caused a resurgence of interest toward population mechanics. Furthermore, J. B. S. Haldane, R. A. Fisher and Sewall Wright have laid the foundations of an exact mathematical theory of population structure. Although their theories represent purely mathematical deductions from established genetic principles and still lack experimental verification, their value should not be underestimated. Indeed, they have provided a guiding light which enables one to state in exact terms many concrete problems to be solved experimentally.

Gene mutations and chromosomal changes are currently supposed to be the mainsprings of the evolutionary process. A rather imposing body of knowledge has accumulated regarding these phenomena as observed under laboratory conditions; relatively little is known about their occurrence and behaviour in natural populations. In no other field is this disproportion more glaring than in *Drosophila* genetics. In the minds of some biologists a false impression has been created, as though a very high mutability under experimental conditions coexists in *Drosophila* with a striking constancy under natural ones. Mutations have been branded "monstrosities", "domestication products" and the like, and their existence in free-living population was doubted or even denied outright.

In a remarkable paper published in 1926, Chetverikov (Tschetwerikoff, 1926) has outlined a programme of studies on the genetics of natural populations of *Drosophila*. Owing to the well-known fact that most mutations are recessive and deleterious for viability, they may exist in natural populations without manifesting themselves except on rare occasions. Hence, a mere inspection of wild individuals is inadequate to detect mutations; genetic methods must be devised to reveal the concealed genetic variability. Only a quantitative study of this variability, taking into consideration factors of geography, ecology, subdivision of populations in semi-independent colonies, etc., can furnish a true picture of population structure.

II. DETECTION OF CONCEALED MUTANTS BY INBREEDING

By far the simplest, although not the most efficient, method for detection of recessive mutants is inbreeding. A fly caught outdoors (such flies are spoken of as "wild" flies, and their chromosomes as "wild" chromosomes) has, of course, two chromosomes of each kind (except for the heterochromosomes). The offspring of a wild female, fertilized in nature by one or more males, carry various combinations of at least four parental chromosomes. By making several pair matings of brothers and sisters, one can, in the second or in later generations raised in the laboratory, obtain individuals homozygous for each of the wild chromosomes present in the wild fly in question. In this way a recessive mutant gene borne in a wild chromosome will become homozygous, and will be detected. In case of the *X*-chromosomes the situation is simpler still, because the sons of a wild female carry either one or the other of the maternal *X*'s, permitting the sex-linked recessives to manifest themselves in the first generation. The practical disadvantages of the inbreeding method are that recessive lethals (except the sex-linked ones) are overlooked, and that unless many pair matings are made some of the wild autosomes may also be missed. Gordon (1936) has given formulae for the evaluation of the data obtained with the aid of this method.

Chetverikov (1927, 1928) has analysed the offspring of 239 wild females of *D. melanogaster* collected at Gelendzhik, Caucasus. His scientific life having been cut short by extrinsic causes, only two preliminary notes of his work have been published. A total of 32 "good" (i.e. having clear external effects) mutants were detected; some of them were allelic to mutants previously known in laboratories,

others were new. Among the latter there was the remarkable mutant "aristapedia", in which the antennae are transformed into leg-like organs. Some mutants were found many times. Thus, the mutation "extra bristles" has appeared in the offspring of nearly 50%, and the mutation "ramuli" in nearly 40% of the wild females. The reviewer finds no statement regarding sex-linked mutants; one may infer that they were not found.

An analysis of 78 females of the same species caught in a certain courtyard in Berlin was made by Timofeeff-Ressovsky (1927), an erstwhile student of Chetverikov. Only 37 females were free of mutants, 26 gave one mutant each, 12 gave two mutants, 2 gave three mutants, and 1 gave five mutants. Ten distinct mutants were recovered, one of which was a sex-linked dominant, one was a sex-linked recessive, two were sex-linked lethals, three were autosomal recessives, and three were autosomal dominants. The presence of sex-linked mutants, including lethals, as well as of autosomal dominants, in the material of Timofeeff-Ressovsky is remarkable. Since these types of mutants can be rapidly eliminated by natural selection, they are expected to be much rarer in natural populations than autosomal recessives or even lethals, and, as will be shown later, they are rare. One may surmise that the stringency of natural selection was temporarily relaxed in the population studied by Timofeeff-Ressovsky, probably because it was rapidly increasing in size due to abundance of food and generally favourable conditions. This surmise is supported by the fact that some of the mutants were found in the offspring of as many as 15 different females (out of the total of 78). This may easily occur in a population which started from very few individuals, and has rapidly grown in size with little selection.

Gordon (1936) analysed 22 *D. melanogaster* females from Slough, England, likewise using the inbreeding method. Eight of the females contained 18 different mutant genes, among which there was a single sex-linked recessive, the rest being autosomal recessives.

It may be noted that Chetverikov, Timofeeff-Ressovsky, and Gordon have found very different sets of mutants in their respective population samples of the same species. This suggests that populations from different localities carry in their collective germ plasms different aggregations of mutants. Such a situation, obviously important from an evolutionary standpoint, agrees well with the theoretical deductions of Wright (1931, 1932); in a species subdivided into non-interbreeding colonies with a small effective size, different groups of mutants may become spread in each colony. Certainly, the data so far reviewed would be insufficient to establish such a conclusion, since the detection of mutant genes producing visible external effects involves too great a "personal equation", and hence much caution is needed in comparing the data of different investigators. Other, and more reliable, data pointing in the same direction are however presented below.

That *D. melanogaster* is not the only species harbouring concealed mutant genes in free-living populations is shown by the studies of Balkaschina & Romaschoff (1935) on *D. phalerata*, *D. transversa* and *D. vibrissina*, Gershenson (1934) on

D. obscura, Gordon (1936) on *D. subobscura*, and Sturtevant, Dobzhansky, and others (see below) on *D. pseudoobscura*. The material of the first three of the above authors came from Zvenigorod, near Moscow, Russia. In *D. phalerata* the offspring of 49 wild females was analysed, and no less than 30 mutants were found (the exact number is indefinite since some probable mutants died before their inheritance could be tested, and others were too erratic in their manifestation). Four of the mutants were found in more than a single line each. In *D. transversa* 22 females were studied; the minimum number of mutants recovered can be estimated as 37, including 8 recurrences of one of them; 8 others were also found more than once each. In *D. vibrissina* 9 females gave 4 mutants. With the exception of a single sex-linked recessive found in *D. transversa*, all other mutants detected were autosomal recessives or very weak semi-dominants. In *D. obscura* 19 females yielded 5 or 6 autosomal recessives, two of which appeared already in the F_1 generation, evidently because the parents happened to be both heterozygous for the respective mutant genes. From a sample of an English population of *D. subobscura* containing 29 females, 28 kinds of mutants were extracted, some of which were found more than once. None were sex-linked or dominant.

III. CENSUS OF MUTANT-BEARING CHROMOSOMES IN *D. MELANOGASTER*

The limitations of the inbreeding method as a tool for the detection of concealed genetic variability have been stated above. More accurate information on the proportion in a population of wild chromosomes carrying all classes of mutant genes, or of definite single mutants, can be obtained with the aid of a different technique. In principle the latter consists in outcrossing wild individuals to a laboratory strain having a chromosome, or chromosomes, with known mutant genes that serve as markers, and thus enable one to follow the course of the particular chromosomes in the inheritance. A series of crosses is so designed that in the end individuals homozygous for the division products of an individual wild chromosome are obtained. The effects of whatever recessives are contained in the wild chromosome in question are thus brought to light through enforced homozygosis. Inverted sections are made use of to suppress crossing-over where needed, and thus to prevent the disintegration of the wild chromosomes in the process of the transmission from generation to generation. A procedure of this sort is illustrated in Fig. 1.

Dubinín and his collaborators (1934) have used the following technique in their studies. Wild females were outcrossed singly to males of the constitution $\frac{\text{Curly } C_{IIL} \text{ } C_{IIR} \text{ } \text{Dichaete Stubble}}{\text{Lobe } l \text{ } C_{IIIL} \text{ } C_{IIIR}}$. The genes Curly, Dichaete, and Stubble are dominant and lethal, or associated with lethals, when homozygous. The "C factors" are inversions suppressing crossing-over in the second (II) and the third (III) chromosomes respectively. The flies appearing in the F_1 generation of the cross, especially the males, are examined to detect the dominant or sex-linked mutants

that may have been carried in the wild female; sex-linked lethals can be detected by the $2\sigma:1\delta$ sex ratio they produce. Then, a single male showing the characteristics of Curly is selected from each culture and backcrossed to females of the same constitution as his mother (i.e. Curly *C/Lobe*, *Dichaete Stubble lC*). The

genetic structure of the Curly males is, evidently,

$$\frac{\text{Wild Chromosome II}}{\text{Curly } C_{IIL} C_{IIR}} \cdot \frac{\text{Wild Chromosome III}}{l C_{IIIL} C_{IIIR}}.$$

In the next generation, males and females showing Curly are again selected and intercrossed. These Curly flies have the same genetic constitution as their father, and carry the division products of a single wild second and a single wild third chromosome that were present in the wild population under investigation. In their offspring, all the non-Curly flies are, therefore, homozygous for a wild second chromosome. If that chromosome carries a lethal, the non-Curly flies fail to appear; mutant genes producing visible external effects cause the non-Curly flies to be abnormal in appearance. Lethals in the third chromosome are not detected in these experiments, but visible mutants show up in one-third of the flies.

Population samples from ten localities in the Caucasus (including Gelendzhik, from which Chetverikov's material had come) and from one locality in central Russia (Tambov) were examined by Dubinin and collaborators (1934, 1936). Their work was on a scale unprecedented and as yet unequalled by any other investigator or group of investigators: 4136 wild second chromosomes, an equal number of thirds, and nearly twice as many *X*-chromosomes were tested. A total of 410 lethals were detected in 3924 wild second chromosomes, so that $10.45 \pm 0.33\%$ of the second chromosomes in free-living populations carry recessive lethal genes. This frequency may seem amazingly high, but in *D. pseudoobscura* even higher concentrations of lethals are observed.

Lethals were found in every population studied (Table I), their frequencies ranging from 7.75% (Piatigorsk) to 21.43% (Armavir). To test the statistical significance of the differences in the frequencies of lethals between various populations, the reviewer has calculated the χ^2 value for the data in Table I involving lethals in the second chromosome (using the Brandt and Snedecor method, cf. Mather, 1938). The χ^2 proved to be 39.3950, which, for 15 degrees of freedom, means that as great or greater heterogeneity may be observed by chance less than once in 100 trials. The conclusion is warranted that the accumulation of lethals in some populations is greater than in others. A similar comparison of the frequencies of lethals on three successive years in the Gelendzhik population (Table I) gives $\chi^2 = 9.9738$, which, for 2 degrees of freedom, is likewise significant.

Not a single sex-linked lethal was found among more than 8000 wild *X*-chromosomes tested. Dubinin *et al.* (1936) have shown theoretically that, since sex-linked lethals are eliminated by natural selection far more rapidly than autosomal ones, the concentration of the former in free-living populations must be only $1\frac{1}{2}$ times greater than the rate of their *de novo* origin by mutation. According to Demerec (1937), the spontaneous mutation rate for sex-linked lethals in most strains of

D. melanogaster is close to 1 : 1000 per generation, and in some strains significantly higher than this. In Dubinin's material 10 or 11 lethals would be, therefore, expected, but none were found (cf. the data of Timofeeff-Ressovsky).

Among the mutants with visible external effects, Dubinin *et al.* have detected a large variety of types, both previously known in laboratories and new ones. The frequency of visibles varies from population to population even more widely than that of the lethals (Table I). In the Delizhan sample no visibles at all were found (this population was tested with the aid of the inbreeding method however), while

Table I. *Frequency of mutant genes in free-living populations of D. melanogaster. (Compiled from the data of Dubinin et al. 1934, 1936)*

Locality	Year	Lethals in chromosome II	Visible mutants in chromosomes II and III	Number of chromosomes II or III tested
Essentuki	1931	20	78	187
	1932	15	25	120
Piatigorsk	1931	8	16	81
	1932	11	35	142
Vladikavkaz	1931	32	35	165
	1932	13	6	115
Mashuk	1931	19	103	180
	1932	15	30	178
Erivan	1931	16	25	102
	1932	7	5	81
Gelendzhik	1933	70	226	877
	1934	78	470	616
	1935	70	410	797
Kislovodsk	1931	13	68	144
Batum	1931	14	23	101
Armavir	1932	9	22	42
Delizhan	1931	Not studied	0	92
Tambov	1931	"	44	120
Total		410	1621	4140

in the Mashuk material for 1931 as many as 103 mutants were detected in 180 second and as many third chromosomes (Table I). Significant yearly variations are likewise recorded for several localities. Although much caution is needed in evaluating these data, since the frequencies of visibles found at different times and by different observers may be subject to grave errors, they are more nearly convincing when taken in conjunction with similar observations on lethals (see above).

Not only the total frequencies of the visible mutants, but also those of individual ones, vary from population to population and from year to year. The mutant "extra bristles" was the commonest in Chetverikov's material collected in Gelendzhik in 1926. Dubinin *et al.* (1936) found it in the same region 102 times among 877 tested females in 1933, 234 times in 616 females in 1934, and 224 times in 797 females in 1935. This mutant is quite common in most other populations studied, and yet it has not been detected in Vladikavkaz and Erivan samples for 1932, although present there the previous year. The occurrence of another common mutant, "comma", is equally erratic. The very extensive data on the status of the Gelendzhik population in three successive years are remarkably interesting, since

they show that the supply of the mutant genes harboured in the germ plasm of a population is in constant flux: some mutants become reduced in frequency or disappear, while others, on the contrary, spread more widely. Unfortunately, the mutants for which the data look most significant are types such as "extra bristles" and "comma", the classification of which is least reliable. But even if a most generous allowance is made for experimental errors, the data remain, in the opinion of the reviewer, rather convincing.

Dubinín and his collaborators (1934, 1936) conclude that the spread, reduction in frequency, or elimination of a mutant in a population is not governed by natural selection alone; restriction of the effective size of the breeding population may cause rather sudden fluctuations of the gene frequencies. Here, then, is experimental confirmation of the theoretical predictions of Wright (1931, 1932), which were also arrived at, in part independently, by Chetverikov (1926), Dubinín (1931), Romaschoff (1931) and Dubinín & Romaschoff (1932) (cf. also Dobzhansky, 1937). Nevertheless, the importance of natural selection as an agent governing population structure is clear enough. It has been pointed out already by Chetverikov (1928) that the concentrations of mutants in free-living populations on the whole tend to be inversely proportional to the degree of the reduction of the viability caused by these mutants. Dubinín and collaborators (1934) have at first doubted the validity of Chetverikov's generalization, but later (1936) considered it as having been confirmed by their own data.

IV. CENSUS OF MUTANT-BEARING CHROMOSOMES IN *D. PSEUDOOBSCURA*

D. melanogaster is now almost cosmopolitan in distribution. It is very commonly associated with man, living partly as a scavenger, and is carried from place to place in commercial products, such as fruit, etc. Exactly where it is autochthonous remains obscure, since the dates of its introduction to various territories are unrecorded, and, moreover, repeated introductions from several sources are very probable. It seems to be at home in the Tropics, and, vice versa, it is certainly not native to the North Temperate Zone. In countries such as the United States and Russia it is never encountered away from human habitations, orchards or gardens. All the genetically analysed population samples of *D. melanogaster* are, then, neither exactly "wild" nor "free-living".

The fact that most animal and plant species which have served as material for genetic investigations might be classed as domestic or semi-domestic has repeatedly been used to cast aspersions on the validity of the resulting data for an understanding of the evolutionary process. To some writers the word "domestication" has become a kind of scarecrow. Since there is no evidence that domestication *per se* either induces or prevents the appearance of any class of genetic changes, this attitude is untenable. In the last analysis domestication is merely a special case of "natural" conditions, this latter term being in reality a name subsuming a great variety of diverse conditions. On the other hand, domestication does modify in some ways the balance of forces acting upon the genetic composition of a population, and

hence cannot be entirely disregarded in studies concerning population dynamics. In the ecology of an animal, even so little "domesticated" as *D. melanogaster*, one can perceive certain special features: temporary relaxations of natural selection caused by overabundance of food and lack of enemies, extreme shrinkages and excessive increases of the population size, introduction or removal from a given locality of masses of individuals by man. Comparison of the population structure in *D. melanogaster* with that in other forms is therefore necessary.

D. pseudoobscura is not much inferior to *D. melanogaster* as a laboratory animal, and is free from the limitations of the latter species. It occurs from British Columbia to Guatemala, and from the Pacific to the Rockies, seldom invading human dwellings or becoming a scavenger, probably because of the competition of *D. melanogaster* and other introduced species. Although its spread by man cannot be entirely excluded, it is at least rare; *D. pseudoobscura* has never been encountered outside the territory in which it is presumably indigenous.

Samples of natural populations of *D. pseudoobscura* were analysed genetically by Sturtevant (1937), who used material from various parts of the distribution area of the species, by Dobzhansky & Queal (1938*b*) with material from isolated mountain ranges in the Death Valley region of California and Nevada, and by Dobzhansky (1939) with material from Mexico and Guatemala. For technical reasons, all these authors studied the gene contents in the third chromosome only, although the species has five chromosome pairs. Only Sturtevant (1937) has made an exploratory study of the second chromosome as well, finding that it seems to behave like the third. The method used by Dobzhansky & Queal for the detection of concealed mutant genes is represented schematically in Fig. 1, while that used by Sturtevant differs from it only in detail. Wild males, or single sons of wild females, are crossed to females homozygous for the third-chromosome recessives orange and purple. In the F_1 a single male is selected and outcrossed to females carrying orange, Blade, Scute, and purple; Blade and Scute are dominants, the former being lethal when homozygous. In the next generation (F_2) females and males showing Blade and Scute are selected and inbred; in the offspring (F_3) two classes of flies appear, namely, wild type and Blade Scute. The whole procedure is evidently designed to obtain individuals homozygous for wild third chromosomes, and the wild types obtained in F_3 satisfy this requirement. Since the gene arrangement in the third chromosome of *D. pseudoobscura* is variable (see below), females of the constitution wild/or *Bl Sc pr* (Fig. 1) usually have an inversion, or inversions that prevent crossing-over. Provided the viability of the wild-type class is equal to that of the Blade Scute, these two classes are expected to appear in the ratio 1 : 2, or 33.3 % of wild type. If, however, the wild third chromosome contains genes decreasing or increasing the viability relative to that of the Blade Scute individuals, the frequency of the wild-type class will fall below or rise above the 33.3 %. Any mutant gene producing visible external effects will be detectable since it will modify the appearance of the expected wild-type class.

A very condensed summary of the results obtained is shown in Table II. In this table there are given the percentages of the wild types obtained in different

cultures, and the numbers of the cultures of each kind. Every culture corresponds, of course, to a single wild third chromosome tested. One may see that the frequencies of wild-type individuals in different cultures vary from 0 to 50%, instead of the theoretical 33.3%. Cultures in which no wild types are produced (the 0% class) are those in which the wild chromosome involved carries a lethal gene. The frequency

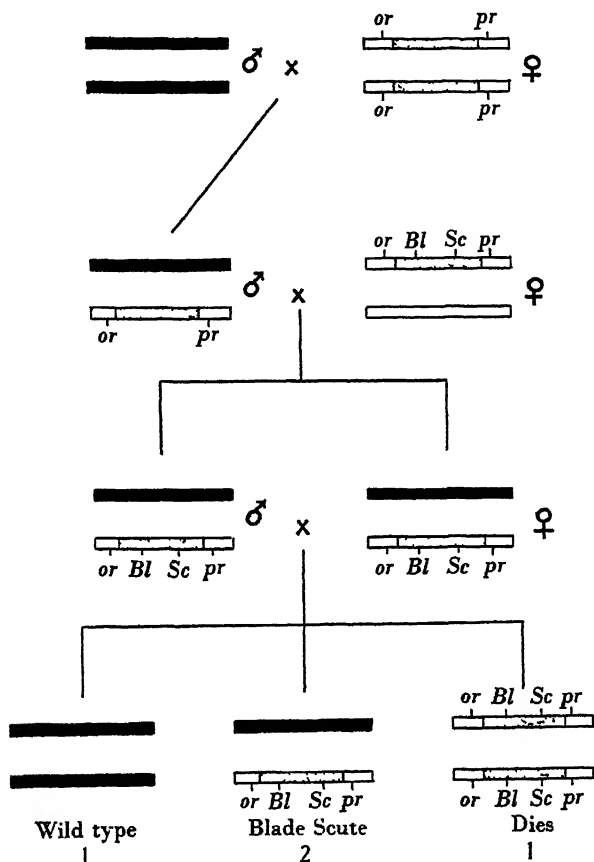


Fig. 1. Experimental procedure for the detection of the genetic variability in the third chromosome of *D. pseudoobscura*. Chromosomes to be tested are shown in black, the tester chromosomes in white, the inverted sections in the tester chromosomes by stippling. (From Dobzhansky & Rhoades, 1938.)

of such chromosomes is $19.25 \pm 1.95\%$ according to Sturtevant (1937), while Dobzhansky & Queal (1938*b*) and Dobzhansky (1939) have found $11.90 \pm 0.75\%$ in the Death Valley region, and $20.83 \pm 2.50\%$ in Mexico and Guatemala. Certain cultures produce some wild types, but far fewer than expected theoretically (Table II). In these cultures the wild third chromosomes carry semi-lethal genes. The distinction between semi-lethals and lethals on the one hand, and between semi-lethals and "normal" chromosomes on the other, is evidently arbitrary, since these

conditions intergrade. Any chromosome that reduces the viability of the homozygote to less than one-half of the norm (which in the experiments under consideration means less than 16% wild type) is classed as containing a semi-lethal. On this basis, $14.96 \pm 0.82\%$ of wild third chromosomes in the Death Valley region contain lethals or semi-lethals, while the corresponding figure for the Mexican populations becomes $28.05 \pm 3.35\%$, for the Guatemalan ones $34.21 \pm 5.19\%$, and for the Sturtevant's sample $19.25 \pm 1.95\%$ (no semi-lethals were found by Sturtevant). Since any fly contains two chromosomes of each kind, the frequency of individuals carrying one or more lethals or semi-lethals is very high in natural populations. In

Table II. *Viability of individuals homozygous and heterozygous for wild third chromosomes (further explanation in text)*

	0	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	20-22	22-24
Death Valley (homozygotes)	91	8	3	3	4	4	2	2	—	—	1	7	11
Mexico (homozygotes)	18	2	—	—	1	—	1	—	1	2	1	—	5
Guatemala (homozygotes)	7	3	—	—	1	—	1	—	1	—	—	—	1
Death Valley (heterozygotes)	—	—	—	—	—	—	—	—	—	—	—	—	—
Mexico and Guatemala (heterozygotes)	4	1	—	—	—	—	—	—	—	—	—	1	6

	24-26	26-28	28-30	30-32	32-34	34-36	36-38	38-40	40-42	42-44	44-46	46-48	48-50	Total
Death Valley (homozygotes)	22	43	66	97	131	93	65	28	20	1	2	2	1	707
Mexico (homozygotes)	5	8	9	6	7	8	7	—	—	—	—	—	—	81
Guatemala (homozygotes)	1	5	5	7	3	2	1	—	—	—	—	—	—	38
Death Valley (heterozygotes)	1	3	7	18	35	44	27	18	12	6	5	—	1	177
Mexico and Guatemala (heterozygotes)	7	20	31	48	63	56	32	24	7	9	4	1	—	314

the Death Valley region only 72.25% of the wild individuals are free of lethals, 25.5% carry one lethal, and 2.25% carry two lethals each. For the Mexican and Guatemalan populations the corresponding figures are 49, 42, and 9% respectively. Individuals carrying two lethals survive, of course, only if these two lethals are not alleles, which is frequently the case. If other chromosomes are infested with lethals to the same extent as the third is, we would be forced to a rather startling conclusion: a decided majority of wild individuals carry one or more lethals or semi-lethals in their germ plasm.

An examination of Table II shows that even if lethals and semi-lethals are disregarded, there is no uniformity in the proportion of the wild types produced in

different cultures. The percentages vary from 16 to 50. To find out whether or not these deviations from the theoretical value of 33.3% are due to sampling errors, the following experiments were made (Sturtevant, 1937; Dobzhansky & Queal, 1938*b*; Dobzhansky, 1939). The Blade Scute individuals from each F_3 culture were intercrossed again, and in the next generation (F_4) the proportion of the wild types was determined. Now, if the variability observed in F_3 is due solely to sampling errors, there should be no correlation between the frequencies of wild types produced in the same line in subsequent generations; a positive correlation would, on the other hand, indicate the genetic modifiers of the viability are involved. In fact, a positive correlation was observed in every experiment. This raises a question: Are these modifiers, if present in homozygous condition, mostly deleterious, or, vice versa, beneficial to the organism? This leads to an even more perplexing question: What is the "normal" viability in *D. pseudoobscura*? In the experiments summarized in Table II the viability of the wild types is measured by comparing their frequency with that of the Blade Scute individuals. But the Blade Scute class is plainly unsatisfactory as a standard of comparison, since the genes Blade and Scute produce rather drastic morphological changes, and hence may be suspected of modifying the viability as well. The following course was adopted by Sturtevant and by Dobzhansky & Queal to obviate this difficulty.

The experimental procedure, shown in Fig. 1, enforces homozygosis for wild third chromosomes. In free-living populations, except the extremely small and inbred ones, there is no similar mechanism in operation, and many or most individuals carry two not very closely related chromosomes of each kind. For organisms like *Drosophila* one may, then, take the viability of individuals resulting from a random combination of gametes present in the population as normal. To simulate this condition as much as possible, wild/Blade Scute individuals coming from different lines, and hence carrying unlike third chromosomes, were intercrossed (cf. Fig. 1). In the offspring, wild type and Blade Scute individuals appeared; here the wild types were heterozygous, instead of homozygous, for third chromosomes (Table II). The frequencies of the wild types vary, of course, from culture to culture, but the distribution curves for the heterozygotes are clearly displaced in the plus direction from those for the homozygotes. This proves that many of the third chromosomes from the natural populations studied carry genes that cause, in homozygous condition, relatively minor deteriorations of the viability. It happens to be relatively simple to determine the average deterioration of the viability produced by homozygosis for a wild third chromosome: disregarding lethals and semi-lethals, this deterioration is about equal to that produced by the combined effects of the mutant genes Blade and Scute. Since mutants as striking as these are in general not expected to survive under natural conditions, the disadvantages of inbreeding and homozygosis are obvious.

An attempt to determine what proportion of the wild third chromosomes carry such unfavourable viability modifiers meets with rather awkward technical difficulties. Without going into detail, it is enough to state that, according to Dobzhansky & Queal (1938*b*), about 39% of the wild chromosomes in the Death Valley region

carry unfavourable modifiers, and this in addition to the 15 % of chromosomes bearing lethals and semi-lethals. On the other hand, some few chromosomes seem to carry genes that, in homozygous condition, enhance the viability of their carrier. If confirmed, this observation may be of value in animal and plant breeding, since the genetic structure of populations in many forms may prove to be not unlike that in *D. pseudoobscura* (Dobzhansky & Rhoades, 1938).

Apart from genes affecting the viability, free-living populations of *D. pseudoobscura* contain many other genetic variants. Dobzhansky & Queal (1938*b*) estimate that in the Death Valley region no less than 3.5 % of wild third chromosomes have mutant genes with visible external effects. This may seem to be a very low frequency compared to those recorded by Dubinin *et al.* (1934, 1936, cf. Table I) in Caucasian populations of *D. melanogaster*. Estimates of the frequencies of visibles are, however, unreliable, especially if made by different investigators. Moreover, the figure of Dobzhansky & Queal does not include certain semi-lethals, which, when the homozygotes survive, might be classed as visibles. Another type of variants are genes modifying the development rate. Such genes are undoubtedly very common in populations of *D. pseudoobscura*, although their exact frequency has not yet been determined. Some preliminary observations suggest that genes causing sterility of the homozygotes are another large class.

Three conclusions are warranted by the data reviewed above. First, a hitherto unsuspected wealth of genetic variability is concealed in the free-living populations of the species of *Drosophila* studied to date. Its concealment is due to the recessiveness of the mutant genes involved. Secondly, a large majority of these mutant genes produce adverse viability effects when homozygous, being in this respect similar to the mutants obtained *de novo* in laboratories. Thirdly, the breeding system in free-living populations resembles, in at least some instances, the so-called balanced lethal condition, until recently considered a genetic oddity. This explains however an old and familiar phenomenon, namely, the deleterious effects of inbreeding and consanguinity.

V. IDENTITY OF LETHALS

Free-living populations of *D. melanogaster*, *D. pseudoobscura*, and undoubtedly of other species as well, are replete with genes deleterious for viability, including a large mass of outright lethals. Wild populations virtually owe their very existence to the recessiveness of the detectable lethals, and to the latter being covered up by their normal alleles. Nevertheless, individuals homozygous for lethals should be produced when two carriers of the same lethal mate. In a very real sense the organism carries the seed of its own destruction within itself. A certain fraction of the population is eliminated in every generation succumbing to the lethals. Yet, the mutation process is unremittingly augmenting the number of lethals already present; their elimination due to homozygosis prevents a complete deterioration of the germ plasm. The degree of the saturation of the population with lethals is determined by the dynamic equilibrium thus created. In general, the elimination rate must be equal to the mutation rate, although this need not necessarily be so in

every generation: seasonal and other cyclical variations may occur. Experimental data bearing on these questions are obviously important for an understanding of population mechanics.

If the frequency of a gene in a panmictic population is q (and of its alleles $1-q$), the frequency of individuals homozygous for it is q^2 . In some populations as many as 34% of the chromosomes contain lethals or near-lethals (the third chromosome of the Guatemalan populations of *D. pseudoobscura*). If all these lethals were alleles, that is if only one kind of lethal were present in the population, about 11.6% of the zygotes formed would be destroyed in every generation. On the other hand, if every lethal were present only once, no elimination at all would take place. The actual situation lies probably between these two extremes. To define it quantitatively, one must know not only the total frequency of all lethals, but also the frequency of each separate one, in other words the allelism of the lethals found.

Dubinín *et al.* (1936) studied the lethals discovered in the second chromosome of *D. melanogaster* from Gelendzhik, Caucasus. In the population sample collected in 1933 there were 70 lethals in 877 tested chromosomes (Table I); of these 51 lethals were studied further. The sample of 1934 had 78 lethals in 616 chromosomes, and all 78 were tested. To analyse the allelism of 78 lethals, every lethal-bearing strain must be outcrossed to every other, which means a minimum of 3003 crosses ($\frac{78^2-78}{2}$). Among the 51 lethals of the 1933 sample, 22 lethals were found once each, 5 twice, 1 three times, and 3 five times; the number of kinds of lethals was therefore 31. In the 1934 sample there were 55 kinds of lethals, 45 of which were encountered once each¹, 5 twice, 3 three times, 1 five times, and 1 nine times.

Since some of the lethals were represented in the Gelendzhik population by more than a single lethal-bearing chromosome, inviable zygotes were being produced in that population. To calculate the frequency of such zygotes, one may recall that in the 1933 sample 70 out of 877, or 8.0%, of the second chromosomes carried a lethal. The frequency of zygotes carrying two lethals, whether allelic or non-allelic ones, must, therefore, be 0.08², or 0.64%. From the data on the allelism it can be computed that about 1 in 33.6, or 2.98%, of zygotes carrying two lethals have two allelic ones. The proportion of the inviable zygotes in the population is therefore 0.0064×0.0298 , or 0.019%. A similar calculation for the 1934 sample gives the following results. The lethal-bearing chromosomes constituted 12.7% of the total, hence the frequency of zygotes carrying two lethals was 1.61%. Of these, 1 in 50, or 2%, bore two allelic lethals, hence 0.032% of all zygotes were inviable. Such calculations were made first by Dr A. H. Sturtevant (unpublished).

The rate of elimination of the second chromosome lethals may now be compared with the data on the rate of origin of new lethals in the same chromosome. The only data bearing on this subject are those of Muller (1928*b*). At 26½–27° C., Muller found 55 lethals in 10,560 chromosome generations, that is, 0.52% of the second chromosomes acquire a new lethal in one generation. At 18–19° C. 14

¹ In the text of the original paper this figure is given as 55, evidently by misprint.

lethals were found in 7012 chromosome generations, making the mutation rate equal to 0.20%. The elimination rate is between 0.02 and 0.03%, i.e. no less than seven times lower than the mutation rate. Although many of the figures on which these computations are based have high probable errors, the discrepancy observed seems to be a real one. At least two possible explanations of this discrepancy may be indicated. First, the computations of the elimination rate are predicated on the assumption that the lethals are completely recessive and are eliminated only when homozygotes are produced. If some of the lethals produce unfavourable effects in heterozygous condition as well, natural selection may eliminate them without homozygotes being produced (suggestion of Dr Sewall Wright, personal communication). Secondly, the elimination rate may be much higher at seasons when the fly populations are reduced to very small size than at seasons when they are flourishing; the samples studied by Dubinin *et al.* were collected at the height of the season (suggestion of Dr A. H. Sturtevant).

The arrays of lethals detected in the Gelendzhik populations in 1933 and in 1934 were compared by Dubinin *et al.* (1936). Among 33 lethals from the 1933 sample and 55 lethals of the 1934 lot, only 9 were identical, the rest having been found either only in 1933 or only in 1934. Among the lethals found in 1933 more than once some were observed repeatedly also in 1934 (one of them nine times), but others were not encountered at all; the same holds for lethals found more than once in 1934. The authors take these facts to prove that spontaneous variations in gene frequencies occur from generation to generation. Although not denying the likelihood of this interpretation being true, the reviewer finds the evidence not entirely convincing. As they stand these data might just as well be used to demonstrate the stability of the gene contents of a population. As corroborative evidence in favour of their interpretation, Dubinin *et al.* adduce the fact that the frequencies of visible mutants in the same population were also observed to vary within rather wide limits from year to year. The observations on visibles are, however, even less convincing than those on lethals. It is also true that Dubinin *et al.* have omitted to state whether the samples collected on successive years were taken in exactly the same locality; this factor may be of consequence, especially in a species dependent on man.

An analysis of the lethals found in wild populations of *D. pseudoobscura* from the Death Valley region (Dobzhansky & Queal, 1938*b*; cf. Table II) was made by Dobzhansky (unpublished). The population samples from which these lethals came were collected in eleven separate localities, but each sample was taken within a rather narrowly circumscribed territory. The study of the lethals and semi-lethals was subdivided in two parts. First, the allelism of lethals from the same sample was studied, to find how common are identical lethals within a presumably panmictic population. Secondly, the identity of lethals from different samples was tested. Although, on the average, only eleven lethals were detected in each locality, in eight of the eleven samples the same lethals were found more than once. Among the 743 test crosses made 22 contained allelic lethals, which gives the frequency of 1:33.8, 2.96% (compared to 2.98 and 2.00% in *D. melanogaster*). The lethals

from population samples collected in different localities are alleles much less frequently: among 5107 test crosses only 20 had identical lethals, the frequency being therefore 1 : 255.3, or 0.39 %. The difference is statistically significant.

This proves that the arrays of lethals borne by populations inhabiting different localities in the same general territory are not alike—a fact of some significance. For in a population of a very large effective breeding size the frequencies of different lethals are determined solely by the mutation rates producing them. If u is the rate of origin of a given lethal by mutation, and if the reverse mutation does not take place, the equilibrium frequency for that lethal in a large population is \sqrt{u} . Since it is very improbable that mutation rates for specific lethals vary widely from population to population, the latter should be more or less alike. The fact that they are not alike indicates that their effective breeding sizes are so small that wide fluctuations in gene frequencies may take place, enhancing or arresting the spread here of one and there of another lethal or a group of lethals. The alternative to this conclusion would be to suppose that the lethals have effects in heterozygous condition, which, acted upon differentially by selection, determine their frequencies in each locality. Although some of the lethals may be slightly dominant, it takes too lively an imagination to postulate different selective agencies in each locality favouring the infestation of the population with a special kind of lethal. It is indeed noteworthy that lethals which are frequent in some populations may be rare elsewhere. Thus, a lethal found four times in 55 chromosomes in a population sample from one of the localities in the Death Valley region has not been encountered in any other sample from the same region (700 chromosomes); another lethal found also four times in a sample of 124 chromosomes has similarly failed to appear in any other sample. The chance that relations such as these may be accidental is negligible.

The rate of elimination of lethals in *D. pseudoobscura* may be computed with the aid of the same method as used for *D. melanogaster*. Since in the populations from Death Valley region 15 % of the third chromosomes carry lethals or semi-lethals, about 2.25 % of the individuals must receive a lethal from each parent. Among these, 2.96 % have two identical lethals, hence inviable zygotes constitute 0.067 % of all zygotes formed. This is a somewhat greater frequency than that observed in *D. melanogaster*. The rate of origin of lethals by mutation is not well known for *D. pseudoobscura*; certain preliminary experiments of Dobzhansky (unpublished) indicate a mutation rate in the third chromosome in the neighbourhood of 0.2 % per generation (at 24½° C.). As in *D. melanogaster*, the apparent elimination rate in *D. pseudoobscura* is much lower than the mutation rate. Possible explanations of this paradox have already been outlined.

VI. OCCURRENCE OF MUTANTS IN NATURE

In examining series of individuals of almost any species of *Drosophila* collected outdoors the impression of a striking constancy is gained. Such variations as there are observed usually involve minor changes in body size, colour, wing venation, and presence or absence of certain bristles. Such changes are as a rule not inherited at all, or else prove to be genetically complex, the complexity being aggravated by

environmental modifiability. No obvious polymorphism, such as is frequently met with in other insects, is apparent. Yet, the abundance of concealed recessive mutants in *Drosophila* populations leads one to expect that mutant homozygotes must be occasionally encountered.

As early as 1911, Lutz found that about 0.34 % of individuals of *D. melanogaster* collected in New York have slight abnormalities of wing venation (extra veins). This character is incompletely recessive, and is subject to influence of minor modifying genes. In *D. repleta* the population consists of two distinct types, namely, a darker and a lighter one (Sturtevant, 1915; Morgan *et al.* 1925). The lighter type behaves as a sex-linked recessive, and is less common than the dark one; nevertheless, it has been recorded in a number of localities: New York, Alabama, Cuba, Arkansas and California. The sex-linked mutant yellow in *D. simulans* was originally found as a single yellow male on a pile of decaying tomatoes in Florida (Sturtevant, 1915, 1929). Morgan (in Bridges & Morgan, 1923) observed differences between wild-type strains of *D. melanogaster* in the intensity of the dark trident pattern on the thorax. The semi-dominant mutant ebony-4 in the same species was detected as a heterozygote among wild flies from South Dakota. Chetverikov (1928) found in the Gelendzhik population of *D. melanogaster* some specimens showing the effects of "extra bristles"; this was indeed expected, since this mutant gene was present, in heterozygous condition, in nearly 50 % of wild flies. The finding of the dominants in the same species by Timofeeff-Ressovskys (1927) shows that they were present as aberrant individuals outdoors. Spencer (1938) found that populations of *D. hydei* from various parts of the United States carry between 3 and 4 % of bobbed flies.

The first systematic attempt to carry out a quantitative study of mutants among wild flies was that of Dubinin *et al.* (1937). These authors have carefully examined more than 129,000 wild individuals of *D. melanogaster* collected in several localities of the Caucasus, in southern Russia, and in one locality in Turkestan. Aside from the minor fluctuating variability, which is difficult to record but which is connected by a series of imperceptible gradations with the more discontinuous type of variation, a large number of clear-cut deviations were found. A serious difficulty encountered in this investigation was that many of the sharply aberrant individuals, simulating mutants, proved to be genetically normal and failed to transmit their characteristics to their offspring. Moreover, and this is more serious still, certain aberrations are dual in nature: some individuals are real mutants with a more or less regular inheritance, while others, externally indistinguishable from the first, are mere phenotypical modifications. Such a simulation of the external characters of mutants by non-genetic abnormalities, termed phenocopies, is at present well known, thanks to the studies of Goldschmidt and others (see a review in Goldschmidt, 1938).

The results of Dubinin, Romashov, Heptner, and Demidova can be summarized briefly by referring to Table III, which represents a selected series of data involving characters that are always, or at least in large part, hereditary. The commonest mutant types are those recessives that are encountered most frequently also in

a heterozygous condition (e.g. extra bristles, analis incompletus). Dominants or semi-dominants are in general not common, but they do occur occasionally (comb of bristles on the thorax). The authors justly emphasize that their data show the effectiveness of natural selection: only those aberrations that produce slight changes in the appearance and the viability of the flies are at all frequent, more drastic alterations being rare or absent. Since for some of these changes the gametic frequencies in several populations are also known (Dubinin *et al.* 1936), it is easy to compute the expected frequencies of homozygotes. Such calculated frequencies are mostly higher than the observed ones, except in cases of presumably "neutral"

Table III. *Some of the mutants and phenocopies detected in wild populations of D. melanogaster by Dubinin et al (1937).*

	Gelendzhik			Archipo-Osipovka			Krymskaya, 1933	Slaviansk, 1933	Der-bent		Buin-aksk		Crimea, 1934	Tashkent, 1934	Odessa, 1934
	1933	1934	1935	1933	1934	1935			1934	1935	1934	1935			
Extra bristles	252	94	15	151	124	40	71	134	10	24	37	37	58	51	29
Small bristles (mosaic)	101	33	4	33	16	10	—	—	1	5	11	15	5	3	5
Bristle comb	19	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Reduced bristles	11	—	—	10	3	—	—	—	—	—	—	—	1	1	—
Mottled eyes	5	1	—	10	—	—	4	2	3	—	—	3	1	1	—
Sepia eyes	2	—	—	—	—	—	—	—	—	—	—	—	1	—	—
Garnet eyes	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dark body (ebony)	3	7	1	2	1	1	14	31	1	—	8	5	8	—	1
Plexus-II	5	10	—	11	6	—	—	33	23	15	18	15	13	6	—
Analis incompletus	16	—	—	50	—	2	8	4	—	—	9	2	9	3	3
Divergent wings	—	1	—	2	15	—	—	2	—	—	—	—	—	—	—
Extra analis	—	8	—	—	11	—	3	7	22	—	6	—	5	1	8
Bright eyes	—	1	—	—	1	—	—	—	—	2	—	—	4	2	—
Notched wings	—	2	—	—	—	—	—	—	—	—	—	3	9	—	—
Abnormal abdomen	—	—	—	—	1	—	—	—	—	—	—	1	2	—	—
Total flies examined	10,000	14,765	6,960	8,925	10,566	6,080	4,604	7,043	3,971	7,088	10,000	8,074	9,189	5,888	7,142

characters. In this connexion an observation of Romashov, quoted in the paper under review, is of interest. In a deep ditch partly filled with rotten fruit Romashov found a population containing a large number of flies homozygous for "divergent wings". When disturbed, the normal flies flew out of the ditch, leaving the divergent-winged ones behind. The authors believe this to be a beginning of the formation of an isolated ecotype.

The arrays of mutants found in different localities are on the whole similar (Table III), even for samples coming from areas so remote as south-west Russia (Odessa) and Turkestan (Tashkent). On the other hand, certain aberrations are distinctly more common in some localities than in others, and apparently significant variations in frequencies are observed from year to year in the same locality.

Despite the difficulties encountered in the classification of some of the mutants and the occurrence of phenocopies, the authors are inclined to believe that these variations indicate a non-adaptive differentiation of the populations due to restriction of the effective size of the latter.

VII. CHROMOSOMAL ABERRATIONS AS MATERIAL FOR POPULATION GENETICS

The process of mutation, using this term in its broadest sense, yields not only gene changes but also alterations of the grosser chromosome structure, such as inversions, translocations, deficiencies, duplications, etc.¹ Among these, deficiencies and duplications alter the genic balance of the individual by removing or by adding blocks of genes, and are apt to produce changes in the morphology and physiology of the carriers. Their effects are similar to those of mutations, but often more drastic in extent. Few deficiencies or duplications are known to occur in natural populations of *Drosophila*, unless some of the lethals will prove to belong to these classes (which is likely in view of the observations of Slizynski (1938) and others).

Inversions and translocations leave both the quantity and the quality of the chromosomal materials unchanged, except when position effects are produced (cf. Dobzhansky, 1936). Translocation heterozygotes are, however, less fertile than the homozygotes, because some of the gametes produced by them give rise to inviable progeny. Since any genetic change becomes established in a population first as a mass of heterozygotes, this property of translocations is bound to interfere with their survival and spread. No translocations have been observed in free-living *Drosophila* populations, although, interestingly enough, species of *Drosophila* do show in a few instances differences of a kind which can be accounted for only on the supposition that translocations have taken place in the phylogeny (Dobzhansky & Tan, 1936). Most of the cytological differences between species of *Drosophila* having, however, arisen through inversion of chromosome segments, it is therefore not surprising to find the same agency responsible for the diversification of the populations within many species as well. Since the introduction by Painter of the technique for studying the chromosomes in the salivary gland cells, the detection and identification of the inversions in *Drosophila* has become easy and accurate. The frequency of certain inversions in free-living populations being greater than that of any one mutant gene, the former become a very valuable tool for investigations bearing on population mechanics.

An additional advantage of inversions is the opportunity they offer to establish, in certain special cases, the phylogenetic relationships of the chromosome structures

¹ Nothing is known about the relative frequency of the origin of these classes of "mutations" in nature. Following an X-ray treatment of the order of 3000 r. units, about one-tenth of the treated X-chromosomes contain newly arisen lethals, and about one-quarter of the treated sperms have detectable chromosomal aberrations (Bauer, *et al.* 1938). The frequency of chromosomal aberrations increases nearly as the square of the number of röntgen units of the treatment, while that of the gene mutations is directly proportional to the treatment, except that the "spontaneous" mutation rate is too high to be accounted for by natural radiation. One may surmise that spontaneous chromosomal aberrations are much less common than mutations.

involved. The method used to accomplish this end has been proposed by Sturtevant & Dobzhansky (1936a) and Dobzhansky & Sturtevant (1938); its essence is as follows. Suppose that an inversion transforms the gene order *ABCDEFGH* into *AEDCBFGH*. This latter gene arrangement may, in turn, be modified by another inversion. The second inversion may include a section of the chromosome lying wholly outside the first (independent inversions, *AEDCBFHG*), or wholly inside the first (included inversions, *AECDBFGH*), or it may overlap the first (overlapping inversions, *AEDGFBCH*). Overlapping inversions deserve special attention. Suppose that in a chromosome of a certain species three gene arrangements are encountered, related as *ABCDEFGH*, *AEDCBFGH* and *AEDGFBCH*. The first of these can, by a single inversion step, give rise to the second, or vice versa. The same relation exists between the second and the third. But the first and the third cannot be so related: the second is a necessary connecting link. Hence, the phyletic relations of the three arrangements can be $1 \rightarrow 2 \rightarrow 3$, or $3 \rightarrow 2 \rightarrow 1$, or $1 \leftarrow 2 \rightarrow 3$, but not $1 \rightleftharpoons 3$.

The above reasoning can be evaded by supposing that changes in chromosomes take place through four breaks arising simultaneously, and through a reunion of the resulting fragments. Multiple breaks are, in fact, no rare occurrence following X-ray treatments, and there is nothing to preclude a similar happening in nature. The consequences of this hypothesis must however be examined further. Following a quadruple breakage, the fragments *A*, *BC*, *DE*, *FG* and *H* may reunite in a variety of ways, namely *AEDGFBCH*, *ADEGFCBH*, *ACBEDGFH*, *AGFBCEDH* and the like. Now, among this array of possible arrangements the arrangement *AEDGFBCH* is unique in that it could be derived from *ABCDEFGH* by two inversion steps involving two breakages each. Others would require more than two such inversion steps, and, more important still, the chromosome must be supposed to break repeatedly at exactly the same points. This is very unlikely, since the distribution of breakages along the length of the chromosome seems to be random (Bauer *et al.* 1938). The decisive fact is that no gene arrangements were found in nature that could be derived from each other only through repeated fractures of the chromosome at the same point. Moreover, the overlapping inversion hypothesis permits prediction of as yet undiscovered gene arrangements; if arrangements denoted above as 1 and 3 exist in nature, it follows that 2 also exists, or has existed. Such predictions were fulfilled on at least two occasions (Dobzhansky & Sturtevant, 1938). Phylogenetic deductions arrived at with the aid of the overlapping inversion method seem to be as secure as inferences about historical events based on experimental evidence ever are.

If the probability of a chromosome breakage at any point is equal to that at any other point, the same inversion is not likely to arise many times in the history of the species. This conclusion is important. Indeed, some inversions are very widely distributed geographically, and are present in tremendous numbers of individuals. Unless a gene arrangement has a positive selective value due to position effects, what agent or agents can bring about such an enormous spread and multiplication of a genetic condition originally present in a single chromosome? A possible

mechanism has been suggested by Sturtevant & Mather (1938). Their premise is that at any given moment and in any given population the gene contents of two, or more, types of chromosomes differing in the gene arrangement are also different. Since deleterious recessives are known to be extremely common in free-living populations, individuals homozygous for a given gene arrangement are somewhat more likely to be homozygous for deleterious recessives than are structural heterozygotes. This puts the heterozygotes at a slight advantage, which may however be sufficient to preserve the rare or newly arisen gene arrangements, and to make them spread further. A corollary to this theory is that an equilibrium condition is reached when all the gene arrangements in a given chromosome become equally frequent in a population. This is seldom the case. The theory of Sturtevant & Mather is not alone sufficient to account for the fate of inverted chromosomes, but it does give a rational explanation of an otherwise puzzling fact, namely, of the spread in natural populations of new gene arrangements which are intrinsically neutral with respect to adaptation.

VIII. INVERSIONS IN *D. MELANOGASTER*

In the early *Drosophila* literature several "C factors" were described, which cause, when heterozygous but not when homozygous, a suppression of crossing-over in the chromosomes in which they lie (Sturtevant 1917, 1919; Bridges & Morgan, 1919, 1923; Morgan *et al.* 1925; and others). It was subsequently found that the "C factors", or most of them, represent inversions of chromosome sections (Sturtevant, 1926, 1931). A majority, if not all, of these inversions were present in strains collected outdoors. X-ray treatments induce new inversions, but their spontaneous origin is extremely rare; the *CIB* inversion of Muller (1928) is perhaps the only one for which such an origin is certain.

The first systematic study of inversions in free-living populations of *D. melanogaster* was made by Sturtevant (1931). While no inversions in the X-chromosome were detected, those in the second and the third proved to be rather common, several different gene arrangements being recorded in each of the latter. No geographical regularity in the distribution of the inversions appeared to be present, which was interpreted as indicating that the same inversions have arisen independently in several regions. In the light of more recent findings it seems more likely that repeated introduction and shifting of flies from region to region by man is the cause.

The introduction of the technique for studying the chromosomes in salivary gland cells has made investigations on inversions and other chromosomal changes found in free-living populations vastly easier than they were before this. Dubinin *et al.* (1936, 1937) have made a large-scale study of *D. melanogaster* populations from various localities in the Caucasus and Turkestan. Larvae were collected in the breeding places, and the cytological study was made in the field. An inspection of the salivary gland cells of each larva gives the information about the structure of two chromosomes of each kind. In some instances larvae were obtained from flies caught outdoors, and the chromosomes of a single larva from each culture were

studied. The gene arrangement present in the chromosomes of a large majority of individuals proved to be the same as that known in most laboratory strains (the "Standard" arrangement). In some populations a fraction of individuals had, however, arrangements different from the Standard. In the *X*-chromosome no inversions were found, although a duplication was discovered in a single individual. In the left limb of the second chromosome an inversion identical with the previously known $C_{II\text{L}}$ Curly was found ($C_{II\text{L}}$ Kutais in author's terminology). In the right limb of the same chromosome two inversions were encountered ($C_{II\text{R}}$ Curly = $C_{II\text{R}}$ Kutais, and $C_{II\text{R}}$ Nova Scotia = $C_{II\text{R}}$ Batum). No aberrations were detected

Table IV. Frequency (in per cent) of second and third chromosomes of *D. melanogaster* carrying inversions (according to Dubinin et al. 1937)

	$C_{II\text{R}}$ Gelendzhik	$C_{II\text{R}}$ Kutais-1	$C_{II\text{R}}$ Kutais-2	$C_{II\text{R}}$ Samarkand	$C_{II\text{R}}$ Batum	$C_{II\text{R}}$ Kutais	$C_{II\text{L}}$ Kutais	
Verni (Alma-Ata)	—	—	—	—	—	—	—	1332
Talgar	—	—	—	—	—	—	—	96
Dzhalal-Abad	2.46	—	—	—	—	—	—	244
Osh	0.30	0.15	—	—	—	—	—	670
Ferghana	0.24	5.36	—	—	—	—	—	1172
Khojent	0.10	1.89	—	—	—	—	—	1006
Samarkand	2.20	—	—	0.32	—	—	—	1226
Bukhara	10.94	5.68	—	—	—	—	—	722
Stalinabad	3.85	6.93	—	—	—	—	—	130
Termez	16.04	—	—	—	—	—	—	374
Ashkabad	3.54	4.71	—	—	—	—	—	764
Baku	0.38	—	—	—	—	0.58	0.58	514
Kachetia	—	—	—	—	—	—	—	426
Gori	—	0.68	—	—	—	0.76	0.34	588
Kutais	—	0.52	0.84	—	—	1.58	1.68	964
Batum	—	—	—	—	0.91	4.14	4.14	410
Suchum	—	—	—	—	—	—	—	492
Erivan	—	—	—	—	—	—	—	324
Gelendzhik	7.40	—	—	—	—	—	—	918
Derbent	2.10	—	—	—	—	—	—	106

in the left limb of the third chromosome, but in the right limb four inversions ($C_{III\text{R}}$) were found, none of which, judging by the descriptions given by the authors, is identical with the previously known ones from American populations. This last point needs confirmation. The frequency of inversions in each chromosome is shown in Table IV.

A geographical differentiation of the population with respect to the inversions is apparent. Thus the inversion $C_{II\text{R}}$ Gelendzhik is found everywhere in southern Turkestan (localities from Dzhalal-Abad to Ashkabad, Table IV), and in northern Caucasus (Gelendzhik, Derbent), but not in northern Turkestan (Verni, Talgar) or in Transcaucasia (Baku-Erivan). On the contrary, the inversions in the second chromosome (C_{II} 's) were detected in Transcaucasia only. Some of the inversions (Kutais-2, Samarkand) seem to be endemics. In detail, the degree of the saturation of populations with inversions is somewhat erratic; localities that are geographically

close may differ more strongly than remote ones. In the Batum population the concentration of the inversions C_{IIL}/C_{IIR} was 4.14% in 1935, and only 0.45% in 1936; the difference is statistically significant. The authors believe that facts such as these indicate that non-adaptive variations in the genetic composition of populations are taking place. It is also possible that each large area has received its original *D. melanogaster* population by importation from a separate source. The minor local and yearly fluctuations may reflect man's influence on the distribution of the species.

IX. INVERSIONS IN *D. PSEUDOOBSCURA*

This species consists of two "races", denoted A and B, that produce sterile male hybrids when crossed. According to Tan (1935) and Koller (1936), the races differ in inversions in either limb of the *X*-chromosome, in the second and in the third chromosomes; the fourth and the fifth chromosomes appear alike. Tan (1935) has also recorded that the gene arrangement is not constant within a race. A more extensive study by Sturtevant & Dobzhansky (1936*a*) and Dobzhansky & Sturtevant (1938) has shown an amazing diversification of the population of both races. The third chromosome is by far the most variable one, eighteen different gene arrangements having been recorded. In the *X*, second, and fourth chromosomes inversions were also found. With the aid of the overlapping inversion method phylogenetic charts showing the descent of the gene arrangements have been drawn. Such a chart for the third chromosome is reproduced in Fig. 2.

In contradistinction to *D. melanogaster*, there is no structural type of the third chromosome of *D. pseudoobscura* which is present in the entire distribution area of the species. The choice of a "Standard" gene arrangement is here purely arbitrary. As such is designated the only arrangement present in both race A and race B (Fig. 2), which, by virtue of this fact, is likely to be the ancestral, or close to the ancestral, arrangement for the whole complex. Another candidate for this distinction is the "Hypothetical" arrangement, which has been postulated theoretically with the aid of the overlapping inversion hypothesis, but as yet not detected in *D. pseudoobscura*. An arrangement possessing the essential properties of the "Hypothetical" one is present, however, in a related species, *D. miranda*.

For purposes of this review a very brief account of the geographical distribution of the gene arrangements will suffice. No one arrangement is present in the entire geographical area of the species, and in no single locality is the entire variety of the gene arrangements to be found. As a rule, each local population has a mixture of chromosomal types, although a territory embracing large parts of Arizona, New Mexico, Utah and Colorado seems to have a uniform population. The greatest variety of arrangements is found in Mexico, Guatemala, California, Olympic peninsula of Washington, and the Rocky Mountains of north central Colorado. Some of the arrangements have oddly discontinuous distributions. Thus, "Tree Line" is found in California, Texas, Mexico and Guatemala, and in the Rocky Mountains; "Estes Park" occurs in east central Mexico and in the Rocky Mountains; and "Olympic" is recorded from the Olympic peninsula, from Mount Whitney in

California, Texas, and in central Mexico. All these relations are undoubtedly deeply rooted in the history of the species, and carry an impress of its migrations and vicissitudes. They are a fascinating material for a zoogeographer and perhaps for a palaeontologist; for the time being they remain insufficiently understood, although Dobzhansky & Sturtevant (1938) have advanced a few speculations concerning them.

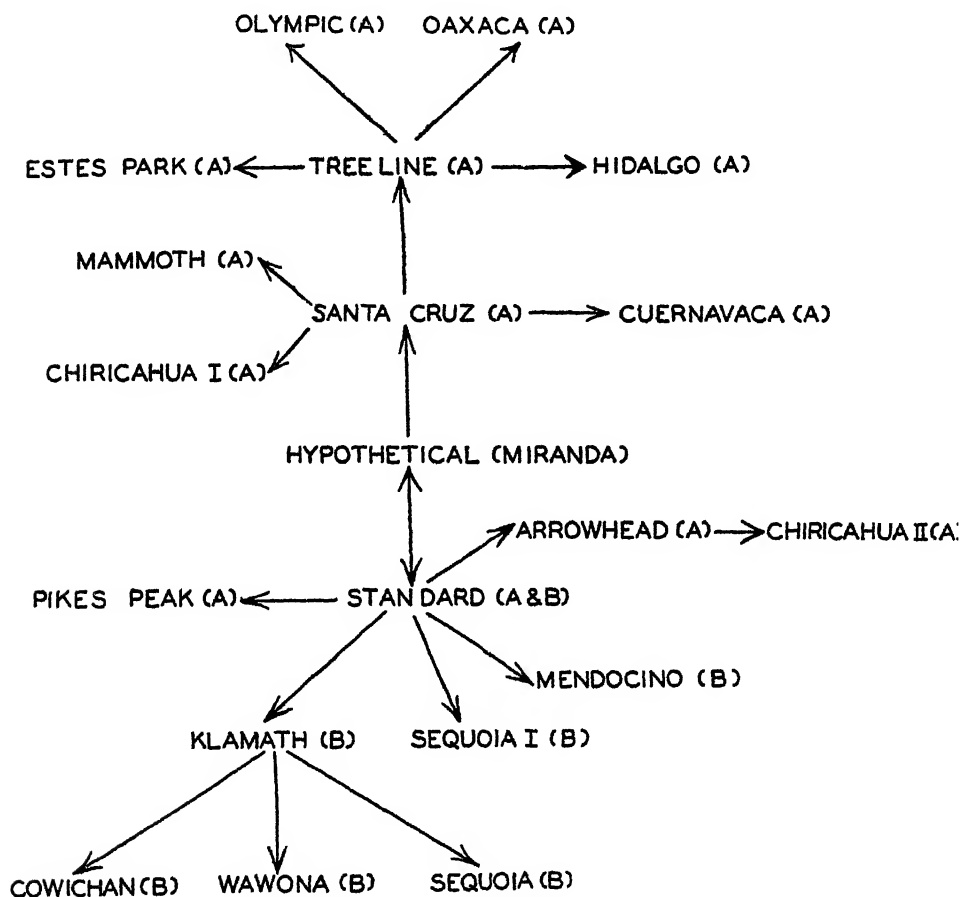


Fig. 2. A phylogenetic chart of the gene arrangements encountered in the third chromosome of *D. pseudoobscura*. Each arrow indicates a single inversion step. The occurrence of the arrangements in race A or race B is indicated in parentheses.

A more exact quantitative study of the frequencies of gene arrangements in a rather limited area was published by Dobzhansky & Queal (1938a). For this study the Death Valley region of California and Nevada was selected. This region is a very arid desert, amidst which rise a number of high and precipitous mountain ranges, most of which are covered, in their upper reaches, with a growth of certain conifers which harbour *D. pseudoobscura*. The intervening stretches of the desert

being unsuitable for this species of fly, the mountain ranges are in effect islands isolated from each other by expanses of uninhabited territory. Population samples were taken on eleven such islands, and the gene arrangements in the third chromosomes were determined cytologically. Table V shows a summary of the results.

Table V. *Frequencies (in per cent) of the gene arrangements in the third chromosome encountered in D. pseudoobscura from isolated mountain ranges in the Death Valley region (after Dobzhansky & Queal, 1938a)*

Mountain range	Arrowhead	Chiricahua	Standard	Mammoth	Chromosomes tested
Lida	76.80 ± 1.80	6.00 ± 1.01	16.80 ± 1.59	0.40	250
Mt Whitney	69.57 ± 4.57	8.70 ± 2.73	21.73 ± 4.10	—	46
Coso	72.27 ± 1.89	14.45 ± 1.48	13.28 ± 1.43	—	256
Cottonwood	51.20 ± 2.13	9.60 ± 1.25	38.80 ± 2.08	0.40	250
Grapevine	50.86 ± 2.23	18.70 ± 1.73	30.43 ± 2.04	—	230
Panamint	67.41 ± 2.11	18.75 ± 1.75	13.83 ± 1.56	—	224
Awavaz	62.20 ± 3.61	19.51 ± 2.95	18.29 ± 2.88	—	82
Kingston	64.08 ± 2.25	5.34 ± 1.06	30.58 ± 2.17	—	206
Charleston	68.75 ± 1.96	19.14 ± 1.66	12.11 ± 1.38	—	256
Sheep Range	88.11 ± 1.53	1.98 ± 0.64	9.90 ± 1.42	—	202
Providence	82.00 ± 1.50	10.00 ± 1.17	8.00 ± 1.06	—	300

Four gene arrangements occur in the Death Valley region, three of them being common in every population. Despite the distances between the mountain ranges being rather small, the populations inhabiting them are often different. Moreover, and this is important, there is no relation between the distances apart of the mountain ranges and the similarity, or dissimilarity, of their populations (cf. a map of the territory involved in Dobzhansky & Queal, 1938a). Neighbouring ranges may be different and remote ones similar. Thus, Panamint, Awavaz, and Charleston are similar, while Kingston, which lies exactly half-way from Awavaz to Charleston, is different from either. Sheep Range and Providence are more nearly similar to each other than either of them is to Charleston, which is geographically intermediate. Migration, that is a diffusion of populations from range to range would evidently lead to a result different from the actually observed one. Neither is there any relation between the ecological conditions on a given range and the characteristics of its population. The only explanation of facts just outlined is that non-adaptive fluctuations in the genetical composition of populations take place, permitting the isolated colonies to differentiate in the course of time. It may be noted here that the presence of inversions in a population does not cause its segregation into separate breeding units; the frequency of inversion homozygotes and heterozygotes is in accord with expectations based on the assumption of the mating being random.

An important development of the above observations was made by Koller (1939), who has studied the frequencies of the gene arrangements in different canyons (gorges) within a single mountain range (Panamint). The populations inhabiting these canyons are at best only partially isolated from each other, since

the forested areas of each canyon are united along the main ridge, as well as over the intervening secondary ridges. Nevertheless, Koller observed about as great a diversification of populations in different canyons as Dobzhansky & Queal have found to exist between separate mountain ranges. The only difference between the situation in the two cases is that adjacent canyons are more nearly similar than remote ones, while no such regularity is observed with mountain ranges. Furthermore, the composition of the population in a single canyon is not constant from year to year; the relative frequencies of the gene arrangements found in the Wildrose canyon of the Panamints by Dobzhansky & Queal in 1937 and by Koller in 1938 are significantly different. A similar shift from year to year was observed in the population of another canyon by Dobzhansky & Sturtevant (1938).

It follows that even within a population spread on an ostensibly continuously habitable terrain colonies may be formed; the amount of interchange of genes between the colonies may be so low that the latter are permitted to differentiate. The sharp yearly variations within a colony suggest that its effective breeding size is very small.

X. INVERSIONS IN OTHER SPECIES OF *DROSOPHILA*

D. melanogaster and *D. pseudoobscura* are not the only species in which inversions were found. In fact, they seem to be present in every species studied in this respect. Sturtevant (1931) detected a cross-over suppressor in the second chromosome of *D. simulans*, which in all probability represented an inversion. Since the introduction of the salivary gland technique the amount of the information available has multiplied at a rapid rate. Kaufmann (1936) described four inversions in the autosomes of *D. ananassae*, two of which were seen only in hybrids between strains of different geographical origin, and two others were present within a strain. One of the inversions appears to be terminal, which is a rare phenomenon. Kaufmann's data were confirmed and extended by Kikkawa (1938), who recovered the four inversions described by Kaufmann and found one new one in material from several localities in Japan and one locality in China. To what extent the population of *D. ananassae* is geographically differentiated remains however obscure. Besides the inversions, Kikkawa describes terminal deficiencies (or duplications) in chromosomes of some strains. Inversions in *D. repleta* and *D. sulcata* (= *D. robusta*) were recorded by Frolova (1936). Her descriptions carry the implication that these inversions arose spontaneously while the strains were bred in the laboratory; it is vastly more probable that they were present in the wild progenitors of these strains (the same remark applies to Koller's (1936) observations on *D. pseudoobscura*).

Dubinín *et al.* (1936, 1937) made rather extensive studies on the populations of *D. funebris* from Moscow, from three localities in Turkestan, and from one locality in the Caucasus. Inversions were found in three out of the four pairs of autosomes present in this species; in one of them four different gene arrangements were established. The Moscow population proved to be the most variable one, the total frequency of structural heterozygotes being as high as 76.3 %. In Turkestan the

Standard type decidedly predominates, while in a very small material from the Caucasus an endemic gene arrangement was discovered. Unfortunately, nothing is known about the extent of the natural distribution of *D. funebris*. In central Russia it is probably native, although very frequently associated with man. It may or may not be native in Turkestan, and the relative uniformity of the Turkestan population may be due to its descent from relatively few introduced individuals. In *D. obscura* and in two other species, as yet undescribed but related to the former, a great variety of chromosome structures was found in a limited material, including multiple inversions of the overlapping type (transpositions according to Dubinin, Sokolov and Tiniakov).

Chromosome variation in *D. azteca* was studied by Dobzhansky & Socolov (1939). The distribution area of this species is broken in two non-contiguous territories, one comprising Mexico and Guatemala and the other a section of central California. In either territory the population contains several distinct chromosomal types. Three out of the five chromosomes present in the species are variable, two of them (autosomes) more so than the third (*X*-chromosome). With the aid of the overlapping inversion method, phylogenetic charts were drawn for two chromosomes. A study of the geographical distribution of the gene arrangements shows that the structural types found in California are more closely related to each other than to the types found in southern Mexico and Guatemala. In north central Mexico (Durango) a population resides which forms a bridge between the Californian and the south Mexican ones.

Inversions were detected also by Dr A. H. Sturtevant in *D. robusta*, *D. melanica*, *D. duncani* and *D. affinis*, by Mr D. D. Miller in *D. algonquin*, and by Mr E. Novitski in *D. athabasca*. The reviewer is obliged to these gentlemen for their kind permission to quote here their unpublished observations. In *D. algonquin* one of the inversions has occurred in a two-armed autosome, and proved to involve the locus of the spindle attachment. This fact is of considerable theoretical interest, since in females heterozygous for such inversions gametes giving rise to inviable zygotes may be produced by crossing-over. Populations in which such heterozygotes occur are, therefore, at a selective disadvantage, not unlike those carrying translocation heterozygotes. The two subspecies of *D. athabasca* (*athabasca typica* and subsp. *mahican*), inhabiting respectively the western and the eastern parts of the United States, differ in multiple inversions in three out of the five chromosome pairs present in the species. Within either subspecies inversions are also found. The reviewer has recently examined the salivary gland chromosomes of the offspring of 52 females of *D. simulans*, collected on the islands of Oahu and Hawaii by Mr Gordon Mainland. The gene arrangement proved to be surprisingly constant in this material; only a single individual was found heterozygous for an inversion in the right limb of the second chromosome, involving the chromosome sections from 44 E to 56 A. It appears that no such inversion is known in *D. melanogaster*, which is a species closely related to *D. simulans*.

XI. THE "SEX-RATIO" FACTORS

Certain males of *D. affinis* were observed to produce offspring consisting of daughters and few or no sons, regardless of the kind of females to which these males were mated (Morgan *et al.* 1925). Gershenson (1928) found the same gene in free-living populations of *D. obscura* from the vicinity of Moscow, and established that it is sex-linked and effective in males only. Females, whether heterozygous or homozygous for the "sex-ratio", produce normal or unisexual progenies depending upon the nature of their mates. Sturtevant & Dobzhansky (1936*b*) observed "sex-ratio" factors in free-living populations of race A and race B of *D. pseudoobscura*, in *D. affinis*, *D. athabasca* and *D. azteca*. A study of this peculiar genetic phenomenon has revealed a number of interesting facts, but, disconcertingly enough, has by no means clarified the whole situation. Nevertheless, for reasons to be stated below, the "sex-ratio" is a very promising material for population studies.

The mechanism of the production of unisexual progenies by "sex-ratio" males is, at least in the case of race A of *D. pseudoobscura*, as follows (Sturtevant & Dobzhansky, 1936*b*). The *X*- and *Y*-chromosomes fail to pair at the meiotic prophase; the *X*-chromosome undergoes one more division than is normally the case, and is clearly quadripartite instead of bipartite; at the first meiotic division the *X* splits, and the two halves, each of them a dyad, pass to opposite poles; the *Y* lags on the spindle and is usually extruded into the cytoplasm; at the second division the *X* splits again, so that all the spermatids carry an *X*-chromosome. The "sex-ratio" behaves as a gene located in the *X*-chromosome, but, at least in *D. pseudoobscura* and *D. azteca*, it is connected with inverted sections preventing crossing-over in its neighbourhood. The nature of this connexion is rather mysterious, especially so since according to newest data of the reviewer the inversions are, in race A of *D. pseudoobscura* and in *D. azteca*, multiple and independent. Yet, the constituent inversions are never found separately.

Gershenson (1928) has pointed out a further peculiarity of the "sex-ratio" factors: a male carrying them transmits them to its entire progeny, while any other sex-linked gene is transmitted to only one-half of the progeny. It follows that, provided the "sex-ratio" gene is not in any way deleterious for its carrier, the frequency of the "sex-ratio" condition in a population must automatically increase, until it reaches 100 %. For a species not capable of parthenogenesis an acute lack of males may mean extinction. Yet, in no population studied is the frequency of "sex ratio" anywhere near 100 %. In race A of *D. pseudoobscura*, for which the data are most extensive, this frequency is not far from zero in the northern part of the species area, and thence rises to about 30 % along the Mexican border. There must exist a mechanism which keeps "sex ratio" under control, but the nature of this mechanism is quite obscure. Since the selection pressure that must exist to prevent an undue spread of the "sex ratio" is very large and rather constantly operative in natural populations, a very promising field for further investigation is open.

XII. SUMMARY

1. The gene mutations and the chromosomal changes obtained in *Drosophila* under laboratory conditions have their counterparts also in free-living populations. The fact that individuals of *Drosophila* found in nature are as a rule homogeneous and only rarely show striking variations is due to the majority of the mutants concealed in them being recessive to the normal or "wild-type" condition.

2. The concealed mutants can be detected either by inbreeding the offspring of wild individuals, or by more complex but more accurate genetic techniques that result in obtaining specimens homozygous for a given "wild" chromosome. As expected, the data obtained with the aid of either method are essentially similar.

3. In *D. melanogaster* the autosomes contain not only mutants producing visible external effects but also many recessive lethals. The kind and the frequency of the mutants found is variable from population to population and from year to year.

4. In free-living populations of *D. pseudoobscura* the third chromosome, and probably other autosomes as well, are infested with lethals, semi-lethals, deleterious viability modifiers, modifiers of the development rate, visible mutants, and other genetic changes. Again, both the quality and the quantity of these changes vary from population to population.

5. Knowing the frequency of allelic lethals within a population, one can compute the rate at which they must be eliminated by natural selection. This rate can be compared to that of the origin of new lethals by mutation. Theoretically, the two rates must be alike in populations at equilibrium. Actually, the supposed elimination rate is smaller than the corresponding mutation rate. This may be due either to some of the lethals not being fully recessive, or else to the population size being subject to sharp seasonal fluctuations.

6. Inversions of chromosome segments are extremely common in free-living populations of all species of *Drosophila* so far studied in this respect. Certain kinds of multiple inversions make it possible to establish the phylogenetic relationships of the chromosome structures involved.

7. In *D. pseudoobscura*, *D. asteca* and probably in many other species each of the different structural types of the chromosomes occurs in a definite geographical region, thus giving rise to chromosomal races.

8. Aside from the above "macrogeographic" variability, there exist also differences, usually of a quantitative nature, between populations inhabiting neighbouring or even contiguous localities. Moreover, the genetic composition of a population does not remain constant from year to year. This "micro-geographic" variability is probably due to a restriction of the effective size of the breeding population in most species of *Drosophila*.

9. A species of *Drosophila* does not represent a single panmictic population, but rather a mass of local colonies that are able to pursue, within limits, different evolutionary courses. The fate of a colony is, of course, controlled by natural

selection, yet selection is not the sole determiner of the population dynamics. As predicted on theoretical grounds by Wright (1931, 1932), shifts in the genetic composition of a population may be due to the limitation of its genetically effective size.

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GRUNDSÄTZLICHES ÜBER TIERSOZIOLOGISCHE AUFNAHMSMETHODEN, MIT BESONDERER BERÜCKSICHTIGUNG DER LANDBIOTOPE

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(Received 10 February 1939)

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I. VORWORT

DIE Beschäftigung mit tiergeographischen und -ökologischen Fragen veranlasste mich in den letzten Jahren immer mehr auch synökologischen Studien nachzugehen. Dabei musste ich, je länger ich auf diesem Gebiete arbeitete, um so deutlicher die Erfahrung machen, dass in der tiersoziologischen Forschung auch in den grundsätzlichen Fragen noch keine Einigkeit besteht. Jeder Forscher, der heute eine tiersoziologische Arbeit schreibt, ist daher gezwungen, zunächst in einer umfangreichen Einleitung seine Arbeitsmethode und die von ihm verwendete Terminologie zu erläutern. Dies ist aber noch nicht einmal die unangenehmste Folge des geschilderten Misstandes, das Bedauerlichste ist die Tatsache, dass es mangels einheitlicher Grundlagen nur schwer oder gar nicht möglich ist, die von den einzelnen tiersoziologischen Forschern erzielten Ergebnisse exakt miteinander zu vergleichen.

Ich habe mich daher in der vorliegenden Arbeit bemüht, einen Weg zu suchen, um zu einheitlichen terminologischen und methodischen Grundlagen in der Tiersoziologie zu kommen. Ich bin mir bewusst, nicht der Erste zu sein, der einen derartigen Versuch unternimmt, aber ich glaube, dass die Zeit heute für eine Klärung der tiersoziologischen Grundfragen reifer geworden ist und hoffe darum, dass die im folgenden gegebenen, wie mir wohlbewusst ist, noch in mancher Hinsicht unvollkommenen Anregungen von anderer Seite aufgegriffen und vervollständigt werden.

II. HISTORISCHER ÜBERBLICK

Die Erforschung der Synökologie der höheren Pflanzen geht in ihren Anfängen weit zurück. Seit A. v. Humboldt ist die Kette der Forscher, die sich mit synökologischen Fragen befasst haben, bis zu Gegenwart nicht mehr abgerissen.¹ Trotzdem hat es lange gedauert, bis ein sicheres, wenigstens in seinen Grundlagen allgemein anerkanntes, methodisches und terminologisches Fundament geschaffen war, auf dem eine im wesentlichen einheitliche pflanzensoziologische Wissenschaft aufgebaut werden konnte. Erst in den letzten Jahrzehnten ist die Pflanzensoziologie aus ihrem ersten, durch methodische und begriffliche Uneinigkeit der einzelnen Forscher gekennzeichneten Entwicklungsstadium in ein zweites, reiferes eingetreten. Einen Markstein auf dem Wege zu dieser reiferen Entwicklungsstufe stellt Braun-Blanquets grundlegende "Pflanzensoziologie" (1928) dar, deren klare und prägnante Darstellungsweise des gesamten pflanzensoziologischen Forschungsgebietes ihre Wirkung auf die synökologischen Arbeiten der letzten zehn Jahre nicht verfehlt hat.

Sicherlich sind auch gegenwärtig noch nicht alle Meinungsverschiedenheiten zwischen den einzelnen pflanzensoziologischen Schulen beseitigt (vgl. Du Rietz, 1921 und Vierhapper, 1924) aber die Verständigung ist doch nunmehr so weit gediehen, dass eine Vergleichung der Untersuchungen verschiedener Autoren ohne weiteres möglich ist.

Vor allem verfügt die Pflanzensoziologie heute für die Zwecke der Klassifikation und Abgrenzung der einzelnen Pflanzengesellschaften über eine allgemein anerkannte pflanzensoziologische Grundeinheit, die Assoziation. Wie die Spezies der abstrakte Sammelbegriff für alle Individuen mit im wesentlichen gleichen Merkmalen darstellt, so bildet die Assoziation den Gesellschaftstypus, dem alle gleichartigen Pflanzenbestände angehören.

Aufgabe der Pflanzensoziologie ist die Analyse der Gesellschaftsstruktur der Vegetation, die Charakteristik und gegenseitige Abgrenzung der Pflanzengesellschaften und die Erforschung der Ursachen ihrer Entstehung und Sukzession. Die Methoden, deren man sich zur Lösung dieser umfangreichen wissenschaftlichen Aufgaben bedient, stimmen heute international so weit überein, dass von verschiedenen Schulen durchgeführte Feldaufnahmen ohne Schwierigkeit miteinander verglichen werden können (vgl. Du Rietz, 1931).

Wie schon einleitend bemerkt, ist die zoologische Schwesterwissenschaft leider noch nicht so weit vorgeschritten. Die Tiersoziologie steht auch heute noch, trotz des regen Interesses, welches in letzter Zeit auch von zoologischer Seite synökologischen Fragen entgegengebracht worden ist, durchaus am Anfang ihrer Entwicklung. Ein knapper historischer Überblick wird das so recht deutlich werden lassen.

Seit der Begründung der tiersoziologischen Forschung durch Meyer & Moebius (1865) und Moebius (1877) ist noch geraume Zeit vergangen, bis die Untersuchung der Lebensgemeinschaften auch auf Seiten der Zoologie in grösserem Umfang in

¹ Einen ausgezeichneten geschichtlichen Überblick über die pflanzensoziologische Forschung seit ihren ersten Anfängen hat Du Rietz (1921) gegeben.

Angriff genommen wurde. Erst zu Beginn des 20. Jahrhunderts wurde beinahe gleichzeitig in Europa und Amerika neuerlich mit der Erforschung der Tiergesellschaften begonnen. In Amerika hat V. E. Shelford mit seiner Schule als Erster tiersoziologische Fragen bearbeitet und seit dreissig Jahren eine grosse Anzahl tiersoziologischer Untersuchungen durchgeführt. Er hat bereits im Jahre 1911 den Satz ausgesprochen: "In general, animal ecology is concerned with the relation of animals to their environments. The first essential is to locate the animal in its environment." Shelford erhebt damit die schon von Moebius (1865, 1877) und Jäger (1874) erkannte weitgehende Abhängigkeit der Tiere von den Umweltverhältnissen zum Hauptgegenstande der tiersoziologischen Forschung. Er verwendet mit dem Pflanzensoziologen Cowles (1901) den Terminus "ecology" als Bezeichnung für die synökologische Wissenschaft im Gegensatz zur autökologischen, ein Gebrauch, der in der amerikanischen Literatur bis heute beibehalten worden ist, sich aber in der übrigen Literatur nicht eingebürgert hat. Eine exakte soziologische Untersuchungsmethode fehlt den ersten Arbeiten Shelfords, wie überhaupt die amerikanische Schule bis heute auch nach dem Erscheinen der zum Teile sehr genauen methodischen Arbeiten von Clements (1916) sich nicht zu restlos exakten Aufnahmsmethoden durchzuringen vermocht hat. Trotzdem dürfen die Arbeiten Shelfords und der übrigen amerikanischen Tiersoziologen nicht unterschätzt werden. Sie sind auf breiter Basis planmässig ausgeführt worden und ermöglichten es Shelford schon im Jahre 1914 in seinem bedeutenden Buche "Animal communities in temperate America" eine fesselnde Schilderung der Tiergesellschaften des gemässigten Nordamerika, besonders der Umgebung von Chicago, zu geben. Wie die amerikanischen Pflanzensoziologen, vor allem Clements (1916), so interessiert auch die dortigen Tiersoziologen in hohem Grade die Frage der Gesellschaftsentwicklung (Sukzession). Shelford kommt auf Grund von in dieser Richtung laufenden Untersuchungen von Waldtiergesellschaften auf sterilem Sand schon im Jahre 1912 zu dem Ergebnis: "The development of forest on sand or other mineral soil is accompanied by an almost complete change of animal mores." Mit dieser Feststellung ist gleichzeitig ein Hinweis auf den engen Zusammenhang der Sukzessionsfolge der Tiere mit derjenigen der Pflanzen gegeben.

In Europa hat Dahl (1904, 1908) als erster Zoologe nach Moebius und Jäger wieder seine Aufmerksamkeit der Erforschung der Tiergesellschaften zugewandt. Er wies darauf hin, dass ein exakter Vergleich verschiedener Lebensgemeinschaften nur möglich sein kann, wenn man deren Tierbevölkerung quantitativ ermittelt. Als Vergleichsbasis schlug Dahl bei Landfängen gleiche Sammelzeiträume vor, eine Methode, die indessen, wie spätere Erfahrungen gelehrt haben, keine verlässlichen Vergleichswerte liefert.

Seit Dahl hat sich zur Bezeichnung tierischer Lebensgemeinschaften der schon von Moebius (1877) eingeführte Terminus Biocönose allgemein eingebürgert. Leider ist der Umfang dieses tiersoziologischen Grundbegriffes schon von Dahl selbst nicht eindeutig festgelegt worden und bis heute in der tierökologischen Literatur schwankend geblieben. Während die Assoziation der modernen Pflanzensoziologie ein klar definierter, dem Artbegriff der Systematik vergleichbarer

Einheitsbegriff ist, wird als Biocönose, um nur ein Beispiel anzuführen, bald der einzelne Baum, bald ein ganzer Wald, also eine Gesamtheit von Bäumen und anderen Pflanzen mit ihren sämtlichen Bewohnern aufgefasst (Dahl, 1921). Dadurch aber wird die Biocönose als Grundeinheit für die Einteilung und Abgrenzung der Tiergesellschaften unbrauchbar.

Es hat nicht an Versuchen gefehlt, diesem Misstand abzuhelpfen. Schon vor zwei Jahrzehnten, als selbst auf pflanzensoziologischem Gebiete noch keine Einheitlichkeit in der Terminologie erzielt war, hat Gams (1918) sich bemüht, eine solche für beide Schwesterwissenschaften herbeizuführen. Seine Bemühungen hatten jedoch wenig Erfolg, nicht zuletzt auch deshalb, weil einzelne der von ihm vorgeschlagenen Begriffe recht kompliziert und zu wenig treffend waren. Ein zweiter Versuch, den Klugh (1923) in der gleichen Richtung machte, blieb wegen seiner völligen Unzulänglichkeit überhaupt unbeachtet. Sehr zu bedauern ist, dass auch Hesses wesentlich klarere Definition des Biocönosebegriffes (1924, S. 343) nicht zu einer inhaltlichen Vereinheitlichung desselben in der späteren Literatur geführt hat. Nach Hesse ist die Biocönose "die Vergesellschaftung von Lebewesen, die einen einheitlichen Abschnitt des Lebensraumes bewohnt und in der Auswahl und Zahl der Arten den durchschnittlichen äusseren Lebensverhältnissen entspricht. Die Glieder der Biocönose sind voneinander abhängig und werden durch den Zustand gegenseitiger Bedingtheit in ein biologisches Gleichgewicht gezwängt, das sich durch Selbstregulation erhält und um einen Mittelzustand schwankt. Neben Arten, die auch anderswo vorkommen, sind in jeder Biocönose gewisse Leitformen vorhanden, die ihr eigen sind." Damit ist bereits der wichtige Grundsatz ausgesprochen, dass die verschiedenen Tiergesellschaften durch ihre Leitformen oder, wie sie Hesse an anderer Stelle nennt, ihre Charakterarten gekennzeichnet sind. Wichtig sind auch die von Hesse geprägten Sätze: "Die Lebensgemeinschaften bilden eine Einheit, deren Glieder sich gegenseitig bedingen. Daher ist es auch nicht angängig, pflanzliche und tierische Bestandteile der Lebensgemeinschaften völlig gesondert zu betrachten; denn die beiden sind aufeinander angewiesen." Hier wird zum ersten Mal die Notwendigkeit enger Zusammenarbeit zwischen Pflanzen- und Tiersoziologie klar begründet, leider ohne sich bis heute im wünschenswerten Ausmasse allgemein durchzusetzen.

Zu einer gewissen Abgrenzung natürlicher Gesellschaftseinheiten in regionalen Ausmassen ist auf zoologischem Gebiete bisher nur die Hydrobiologie gelangt. Hier hat nach den viel zu wenig beachteten, tiersoziologisch äusserst interessanten Untersuchungen Ekmans (1915) die bahnbrechende Entdeckung Thienemanns (1925), dass die Chironomidenlarven des Typus *Tanytarsus* beziehungsweise *Chironomus plumosus* ganz verschiedene Seentypen (nährstoffreiche beziehungsweise nährstoffarme) bewohnen, zu einer Einteilung der Binnenseen nach Leitformen der Bodenfauna geführt. Bemerkenswerte Versuche in dieser Hinsicht haben u.a. Alm (1922) in Schweden, Lundbeck (1926) in Norddeutschland, Valle (1927, 1928) in Finnland, Lenz (1928) im Hochgebirge Norwegens und Decksbach (1928) in Russland unternommen. Alle diese Untersuchungen zielen jedoch mehr auf eine Charakterisierung der Seentypen als auf eine gegenseitige scharfe

Abgrenzung der wohl in den meisten Seen in grösserer Anzahl vorhandenen Tiergesellschaften ab. Eine exakte Abgrenzung dieser ist meines Wissens bisher in der Limnologie noch nicht versucht worden und dies ist wohl der Grund, weshalb die Versuche einer Charakterisierung der Seen auf Grund ihrer Fauna bisher noch zu keinem restlos befriedigenden Ergebnis geführt haben.¹

Trotzdem ist es der Hydrobiologie jedoch, wie u.a. die Arbeiten von Thiene-mann (1925 *a*, *b*), Naumann (1932) und Ruttner (1937 *b*) zeigen, vorwiegend auf produktionsbiologischer Grundlage gelungen, Einblick nicht nur in das Gesellschafts-gefüge sondern auch in den Gesellschaftshaushalt und in die Gesellschaftsentwick- lung vieler Süsswasserbiocönosen zu gewinnen.

In der Untersuchung von Landtiergesellschaften stellt eine Arbeit von Dogiel (1924) einen bemerkenswerten Fortschritt dar. Der genannte Autor hat wohl als erster Tierökologe planmässig die Tierwelt gleichgrosser Vergleichsflächen (400 cm²) quantitativ aufgesammelt und miteinander verglichen. Er kam bei seinen innerhalb eines grösseren Wiesenbiotops durchgeführten Aufnahmen zu dem Ergebnis, dass die auf Flächen gleicher Vegetationszusammensetzung gesam- melten Proben eine qualitativ wie quantitativ weitgehend übereinstimmende Zusammensetzung des Tierbestandes aufwiesen, während die von Flächen ver- schiedener Vegetation stammenden Proben sowohl hinsichtlich der Artenzusam- mensetzung als auch hinsichtlich der Individuenzahl erhebliche Unterschiede zeigten. Leider hat Dogiel auf Grund seiner Untersuchungen keine genaueren Aufnahmen zum Zwecke einer exakten Charakterisierung der einzelnen von ihm festgestellten Tiergesellschaften gemacht, sondern sich mit einer produktions- biologischen Auswertung der erzielten Resultate begnügt. Die Methode der quantitativen Aufsammlung des Kleintierbestandes auf Flächen gleicher Grösse ist seit Dogiel noch von verschiedenen anderen Autoren angewandt worden. Krogerus (1932) und Brundin (1934) sammelten in Flächenquadraten von 1 m² die Arthro- poden- beziehungsweise nur die Coleopterenfauna möglichst quantitativ auf. Wcese (1924) und Shackleford (1929) bezogen ihre allerdings weniger exakten Aufnahmen auf die gleiche Flächeneinheit. Oekland (1929) verglich Schnecken- bestände, die er auf Flächen von 25 mal 25 cm auf der Bodenoberfläche und in den obersten Bodenschichten quantitativ aufgesammelt hatte. Morris (1922, 1927) und Frenzel (1936) führten auf Flächen von 10 mal 10 beziehungsweise 25 mal 25 cm quantitative Aufsammlungen der Mikrofauna des Bodens durch, wobei sie die grösseren Flächen ausschliesslich nach mit freiem Auge sichtbaren grösseren Tieren durchsuchten, die mikroskopische Milben- und Nematodenfauna dagegen nur in den 100 cm²-flächen feststellten. Grundsätzliche Studien darüber, wie gross Probeflächen sein müssen, damit sie den Artenbestand einer Tiergesellschaft einigermaßen vollständig umschliessen, wurden bisher meines Wissens noch nicht ausgeführt.

Für die Entwicklung der Tiersoziologie haben einige in den letzten 15 Jahre

¹ In den letzten Jahren hat Rzoska (1936) darauf hingewiesen, dass eine genauere Gliederung der verschiedenen in den litoralen Bezirken der Seen vorhandenen Tiergesellschaften für die Seetypen- lehre von grossem Werte sein könnte.

erschienene amerikanische Arbeiten erhebliche Bedeutung. In einer Arbeit von Weese (1924) und ganz besonders in einer solchen von Shelford & Towler (1925) wird erstmalig der Versuch unternommen, Tiergesellschaften in exakter Weise durch bestimmte Tierarten zu kennzeichnen. Zur Gesellschaftscharakteristik werden die hinsichtlich ihrer Individuenzahl dominierenden Arten verwendet. Shelford & Towler stellen die Forderung auf: "Communities must be determined by dominants rather than habitat; the limits of the dominants as such are the limits of the community." Diese Methode nähert sich merklich der in der modernen Pflanzensoziologie, besonders von der schwedischen pflanzensoziologischen Schule (vgl. Du Rietz *et al.* 1920) angewandten. Von den amerikanischen Tiersoziologen wird in gleicher Weise wie von den schwedischen Pflanzensoziologen nicht der Treuegrad sondern die Dominanz der einzelnen Arten zur Grundlage für ihre Verwendung als Charakterarten des Gesellschaftstypus gemacht.

Den meines Wissens neuesten Stand der amerikanischen tiersoziologischen Aufnahmepaxis gibt eine Arbeit von Shackleford (1929) an. Shackleford ging bei Untersuchung von Wiesengesellschaften in Zentral Illinois so vor, dass er an 9 verschiedenen Stationen innerhalb eines ursprünglichen Prairiestreifens, im Laufe eines Jahres zu wiederholten Malen Aufsammlungen von Tieren vornahm. Er versuchte die Fauna der Grasschicht annähernd quantitativ zu vergleichen, indem er die durch 5 Schläge mittels eines Kätchers von 30 cm Durchmesser gefangenen Tierbestände miteinander verglich. Ausserdem stellte er die Fauna der Bodenoberfläche auf einer Fläche von $\frac{1}{2}$ Quadratfuss annähernd quantitativ fest und rechnete das Ergebnis auf 1 m² um. Es ist klar, dass bei Anwendung dieser Methode nur sehr bedingt vergleichbare Werte erzielt werden konnten und dass dabei vor allem die Mikrofauna des Bodens unberücksichtigt blieb.

Einer eingehenden Erörterung bedarf der von Shackleford in Anlehnung an Shelford und Clements zur Charakterisierung der Tiergesellschaften eingeschlagene Weg. Shackleford nennt die sichtlich von der Vegetation abhängigen Kleintiergesellschaften der von ihm untersuchten Prairie "Presocieties" und benennt sie nach den Tierarten, die in entsprechender Individuenzahl, Körpergrösse und möglichst ausgedehnter Teilperiode des Jahres in der Gesellschaft (Community) vorkommen. Die während des grössten Teiles des Jahres zahlreich als vollentwickelte Tiere vorhandenen Arten nennt er "Predominants". Neben diesen unterscheidet er "Seasonals", das sind solche, die nur während einer begrenzten Periode des Jahres häufig und somit für bestimmte jahreszeitliche Aspekte (Seasonal societies) charakteristisch sind. Es ist das Verdienst der amerikanischen tiersoziologischen Schule die jahreszeitlichen Unterschiede in der Zusammensetzung der Tiergesellschaften erstmalig eingehender untersucht und beschrieben zu haben. Shackleford unterscheidet in den von ihm untersuchten zwei Presocieties der trockenen beziehungsweise nassen Prairie je 5 "Biotic seasons", 5 Perioden während welcher in jeder der beiden Presocieties eine bestimmte "Seasonal society" (prevernal, vernal, estival, autumnal und hiemal societies) vorhanden ist. Jede "Biotic season" ist gekennzeichnet durch das Vorhandensein spezifischer, in der betreffenden Saison sehr häufiger "Seasonals" und manchmal ausserdem noch durch das Anwachsen der

Abundanz gewisser "Predominants". Für die Rangordnung der Predominants und Seasonals ist die relative Abundanz, Grösse und Lebenszeit massgebend, deren Masstab der Influenzgrad darstellt. Ob eine Art als "influent" oder "subinfluent" bezeichnet wird und an welcher Stelle sie innerhalb der influenten beziehungsweise subinfluenten Arten gereiht werden muss, hängt davon ab, welcher Influenzgrad ihr zukommt. Massgebend für diesen ist nicht die absolute Abundanz sondern die relative, das heisst die relative Häufigkeit im Vergleiche zu den übrigen Tiertarten der Gesellschaft während der gleichen Zeitperiode. Shakleford ist bemüht durch die Einführung der Klassifikation nach Influenzgraden, die er aber nicht zahlenmässig ausdrückt, die Artenlisten, die bei der Gesellschaftscharakteristik aufgestellt werden müssen, zu vereinfachen. Er sucht damit die zahlenmässige Angabe der Abundanz, der Zeitdauer des Auftretens der einzelnen Arten, der Grösse und der Bedeutung für die betreffende Gessellschaft zu ersparen, kann aber doch nicht verhindern, dass durch Weglassung dieser Daten seine Angaben ungenauer werden.

Betrachtet man die von ihm unterschiedenen "Presocieties" der trockenen und nassen Prairie eingehender, so sieht man bald, dass sie nicht dem Assoziationsbegriff sondern eher dem Formationsbegriff der europäischen Pflanzensoziologen entsprechen. Dadurch ist es auch zu erklären, dass die verschiedenen in der nassen Prairie untersuchten Stationen keine völlig übereinstimmende Faunenzusammensetzung besitzen. Die Untersuchung einer grösseren Zahl von Probeflächen und die stärkere Berücksichtigung der Vegetationsunterschiede würde es zweifellos ermöglicht haben, innerhalb der nassen Prairie weitere Gesellschaftseinheiten auszuscheiden und damit die Presocieties dem Assoziationsbegriff der europäischen Soziologen anzugleichen.

Beachtlich ist bei Shakleford wie überhaupt bei den amerikanischen Tiersoziologen der Versuch, nicht nur die Kleintiere sondern auch grössere Landtiere in der Gesellschaftsanalyse mitzubetrachten und ihrer Bedeutung für den Gesellschaftshaushalt durch Kombination des Abundanzbegriffes mit der Körpergrösse bei Festlegung der Rangordnung der einzelnen Arten innerhalb der Gesellschaft Rechnung zu tragen. Bisher ist es allerdings noch nicht gelungen, eine exakte Stufengliederung der Landtierwelt nach ihrer Körpergrösse durchzuführen.

Den ersten wirklich exakten Versuch grössere Landtiere durch quantitative Aufnahmen hinsichtlich ihres soziologischen Verhaltens zu studieren haben finnländische Ornithologen unternommen. Tiersoziologisch bemerkenswert ist vor allem eine Arbeit von P. Palmgrén (1930), weil darin auf Grund quantitativer Quadrataufnahmen (100 mal 100 m) eine deutliche Abhängigkeit der Vögel trotz ihrer grossen Beweglichkeit in ihrer räumlichen Verteilung von bestimmten Umweltbedingungen nachgewiesen wird. Als besonders wichtigen Umweltfaktor spricht Palmgrén die Vegetation an, die er in Anlehnung an die Waldtypenlehre Cajanders (1909, 1926) charakterisiert und zur Grundlage der Biotopabgrenzung macht. Die in den letzten zehn Jahren veröffentlichten tiersoziologischen Arbeiten skandinavischer Autoren sind sämtlich stark von den Untersuchungen Palmgréns beeinflusst und schliessen sich ihnen methodisch in vieler Hinsicht an.

In den letzten Jahren ist die Zahl der Arbeiten, die sich vorwiegend oder doch

teilweise mit tiersoziologischen Fragen befassen, erfreulicher Weise derart angestiegen, dass es nicht mehr möglich ist, auch nur die wichtigeren von ihnen zu besprechen. Auf die bedeutenderen Publikationen ist im Literaturverzeichnis hingewiesen. Im folgenden seien nur noch drei Arbeiten eingehender erörtert, die für den derzeitigen Stand der Tiersoziologie in Europa besonders kennzeichnend sind, die drei grossen tiersoziologischen Monographien von Krogerus (1932), Brundin (1934) und Renkonen (1938).

Die Studie von Krogerus über die Tribsandarthropoden Finnlands stellt eine erste, den Forderungen der modernen Tiersoziologie entsprechende monographische Bearbeitung einer Gruppe von Landtiergesellschaften auf der Grundlage quantitativer Flächenaufnahmen dar. Krogerus geht von der Zoocönose als tiersoziologischem Einheitsbegriff aus. Eine Zoocönose ist nach seiner Definition "ein den gesamten Tierbestand des Standortes umfassendes, sich selbst regulierendes Bevölkerungssystem von Tieren, die durch eine durch soziologische Affinität ihrer Hauptmitglieder charakterisierte Artengruppe zusammengehalten werden". "Die kleinen Tiergesellschaften innerhalb der Zoocönose, die durch die gleichen unentbehrlichen ökologischen Bedingungen zusammengehalten werden, die aber oft etwas Zufälliges an sich haben", nennt Krogerus mit Friederichs (1930) "Konnexe". Die soziologische Charakteristik der Kleintiergesellschaften beruht nach dieser Definition auf der Feststellung einer für den betreffenden Gesellschaftstypus kennzeichnenden "durch soziologische Affinität ihrer Hauptmitglieder charakterisierten Artengruppe", also wie bei Shelford und Towler auf der Zusammensetzung des Tierbestandes selbst; eine scharfe Abgrenzung von Zoocönosen und Konnexen wird leider nirgends gegeben. Eine enge Anlehnung der Tiersoziologie an die Pflanzensoziologie lehnt Krogerus mit der Begründung ab, dass wir in den Tiergesellschaften wegen der geringeren Raumkonkurrenz der Tiere im Vergleiche zu den Pflanzen und der grösseren Vagilität derselben nicht die gleiche feste Gesetzmässigkeit erwarten dürfen wie in den Pflanzengesellschaften. Diese Behauptung wird allerdings nicht weiter bewiesen, die Ergebnisse der Untersuchungen zeigen vielmehr, dass im Bereiche der Tribsandgebiete auch innerhalb der Tiergesellschaften hinsichtlich ihrer Zusammensetzung eine klare Gesetzmässigkeit besteht. Krogerus unternimmt im synökologischen Teile seiner Arbeit sogar selbst den Versuch, die Konstanz der für die Tribsandgebiete charakteristischen Tierarten nach der Methode der Schweizer pflanzensoziologischen Schule zu bestimmen. Er untersucht, in welchem Prozentsatz einer Anzahl typischer, quantitativ untersuchter Probeflächen von 1 m² Grösse die einzelnen Arten vorkommen und teilt sie nach den erhaltenen Prozentzahlen in konstante (über 50%), akzessorische (25–50%) und akzidentielle (unter 25%) Arten ein. Das Ergebnis der Untersuchungen ist, dass jedem einzelnen von 9 Lebensraumtypen (Biochorien), die Krogerus in den Tribsandgebieten Finnlands unterscheidet, seine eigenen, regional konstanten Arten zukommen. Unter den konstanten Arten überwiegen die monophagen Pflanzenfresser. Ausser der Konstanzbestimmung versucht Krogerus auch Dominanz- und Treuegradbestimmungen. Hinsichtlich des Treuegrades unterscheidet er mit Hesse eucöne, tyhocöne und xenocöne Arten;

als eucön bezeichnet er stenotope Leitformen oder Charakterarten, die den Lebensbedingungen eines Biotops derart eng angepasst sind, dass sie an anderen Plätzen nicht zu leben vermögen. Krogerus gibt insgesamt 127 eucöne Arthropodenarten für die von ihm untersuchten Tribsandgebiete an. Diese 127 Arten sind ohne weiteres als Charakterarten der die Tribsandgebiete bevölkernden Tiergesellschaften anzusprechen, sofern ihnen ein entsprechender Konstanzgrad (Stetigkeit) zukommt. In der Tat ist aus den in der Monographie von Krogerus enthaltenen Tabellen zu entnehmen, dass eine ganze Reihe der als streng eucön bezeichneten Arten auch einen hohen Konstanzgrad aufweist. So besitzt die eucöne Fliege *Tetanops myopina* nach Krogerus im Bereiche der Dünenhügel mit *Elymus*-vegetation eine Konstanz von 92,3% und der Käfer *Hesperophilus arenarius* in den Salinen eine solche von 86%. Es erscheint unter diesen Umständen verwunderlich, warum Krogerus nicht auf Grund des von ihm zusammengetragenen, äusserst wertvollen synökologischen Tatsachenmaterials an die exakte Beschreibung der verschiedenen Gesellschaftstypen innerhalb der finnländischen Tribsandgebiete auf Grund des Artenbestandes geschritten ist.

Im Gegensatz zu Krogerus haben Brundin (1934) und Renkonen (1938) nicht alle Arthropoden sondern nur die Coleopteren bei ihren soziologischen Untersuchungen berücksichtigt. Sie haben damit nur einen kleinen Ausschnitt aus den tatsächlichen Tiergesellschaften erfasst, nur Teilbestände und so zweifellos eine ganze Reihe charakteristischer Arten übersehen und überhaupt keinen vollständigen Einblick in die Struktur der untersuchten Gesellschaftsverbände gewonnen. Trotzdem sind die Arbeiten beider Autoren wegen der angewandten Untersuchungsmethoden und der erzielten Ergebnisse von allgemeinem Interesse und müssen darum hier besprochen werden. Sowohl Brundin als auch Renkonen sind nicht auf die Erzielung absolut quantitativer Proben ausgegangen, sondern begnügten sich mit Annäherungswerten, in der Erkenntnis, dass die verfügbaren Sammelmethoden heute eine wirklich quantitative Aufsammlung der Kleintierwelt, ja selbst nur der Arthropoden, innerhalb grösserer Flächen nicht ermöglichen.

Brundins grosses Verdienst ist es, als erster Tiersoziologe konsequent den Versuch einer tiersoziologischen Aufnahme auf pflanzensoziologischer Grundlage durchgeführt zu haben. Auch andere Forscher, wie Weese (1924), Shackleford (1929), Palmgrén (1930) und Krogerus (1932) sind bei ihren tiersoziologischen Untersuchungen schon von den Pflanzengesellschaften ausgegangen, keiner dieser Forscher hat aber die in seinem Untersuchungsgebiete vorhandenen Phytoassoziationen genau studiert und seine Aufsammlungen so vorgenommen, dass jede von ihnen einwandfrei nur aus einer einzigen Pflanzenassoziation stammte. Dies hat nun Brundin getan und dadurch die Möglichkeit erhalten, einwandfrei festzustellen, dass in seinem allerdings nicht sehr ausgedehnten Untersuchungsgebiete die einzelnen Assoziationstypen hinsichtlich der sie besiedelnden Tiergesellschaften erhebliche Unterschiede aufwiesen. Leider ist Brundin auf dem sicherlich richtigen Wege einer engen Zusammenarbeit zwischen Pflanzen- und Tiersoziologie etwas zu weit gegangen. Er baut seine Untersuchungen auf die stillschweigende Voraussetzung auf, dass sich Zahl und Grenzen der Tiergesellschaften mit denen der

einzelnen Pflanzenassoziationen decken, eine Annahme, die zwar in den meisten Fällen zutreffen dürfte, aber doch in jedem Einzelfall nachgeprüft werden muss. Es könnte ja auch sein, dass einzelne Tierarten einer Assoziation feiner auf die Umweltfaktoren reagieren als die mit ihnen zusammenvorkommenden Pflanzenarten und dass demnach innerhalb einer einheitlichen Phytoassoziation mehrere Tiergesellschaften zur Ausbildung gelangen. Es muss also die schon von Shelford & Towler (1925) erhobene Forderung, dass die tiersoziologische Forschung immer wieder von den Tiergesellschaften selbst auszugehen habe, im Gegensatz zu Brundin unbedingt beibehalten werden. Ebenso muss daran festgehalten werden, dass die Tiersoziologie in erster Linie die Gesellschaftsbeziehungen der Tiere zu untersuchen, sie zu beschreiben und kausal zu erklären hat. Brundin geht bei seinen Untersuchungen vom Standort, als dem Lebensmedium eines Tieres oder einer Tiergesellschaft aus, wobei sein Standortsbegriff auch die Vegetation mit einschliesst. Demgemäss bezieht er seine äusserst wertvollen Dominanz- und Stetigkeitsuntersuchungen nicht auf die einzelnen Gesellschaftsverbände selbst sondern auf die Standortstypen und spricht im Gegensatz zu Braun-Blanquet nicht von Gesellschafts- sondern von Standortstreue. Er verfällt damit in einen Gedankenfehler, den auch viele Pflanzensoziologen gemacht haben. Der Kausalzusammenhang zwischen Pflanzengesellschaften und Standortstypen ist, wie besonders die schwedische pflanzensoziologische Schule gezeigt hat, kein so direkter wie vielfach angenommen wurde. Pflanzengesellschaften sind nicht nur von den Standortsfaktoren sondern auch von der Kampfkraft der einzelnen an diesen Standorten lebensfähigen Arten sowie von der Entwicklungsgeschichte der einzelnen Assoziationen abgänglich. Es können aus diesem Grunde ohne weiteres an gleichartigen Standorten mehrerer Pflanzengesellschaften zur Entwicklung gelangen oder aber umgekehrt an Standorten mit etwas abweichenden Umweltbedingungen trotzdem dieselben Pflanzengesellschaften stehen. Das Gleiche kann natürlich auch bei Tiergesellschaften der Fall sein, wie Shelford schon 1914 gezeigt hat. Der Satz Palmgréns (1930): "Man dürfte nicht leugnen können, dass die Klassifikation der Vegetation auf Grund der Vegetation selbst auf einem sichereren Boden ruht als die Einteilung auf Grund des Standortes" gilt *mutatis mutandis* auch für die Tiersoziologie.

Trotz der erhobenen Einwände darf aber die Bedeutung der Untersuchungen Brundins für die Weiterentwicklung der Tiersoziologie nicht verkannt werden. Brundin hat als erster Tiersoziologe versucht den Biocönosebegriff in seinem Umfange dem pflanzensoziologischen Einheitsbegriff, der Assoziation, anzugleichen. Seine soziologische Grundeinheit ist wie bei Krogerus die Zoocönose, die er auch ähnlich wie dieser Forscher definiert. Trotz der gleichen Definition ist aber der Umfang der Zoocönose bei Brundin dem der Zoocönose bei Krogerus nicht völlig gleich, der Brundin'sche Zoocönosebegriff ist vielmehr enger gefasst und schärfer umgrenzt. Dies zeigt sich mit aller Deutlichkeit, wenn man den speziellen Teil der besprochenen Arbeiten beider Forscher miteinander vergleicht. Brundin hat sein gesamtes Untersuchungsgebiet, die Umgebung des Torneträsk, konsequent in Standorte gegliedert, die im grossen Ganzen der Assoziationseinteilung der

Pflanzensoziologie entsprechen, und nahezu für jeden Standort standortstreu Charakterarten unter den Käfern feststellen können. Er hat damit erstmalig in grossem Umfange den Nachweis geführt, dass auch zahlreiche Kleintiere, nicht nur viele Pflanzen in einer hohen Masse standorts- beziehungsweise gesellschafts- gebunden sind. Dieses wichtige Ergebnis wäre noch deutlicher zum Ausdruck gekommen, wenn Brundin wie Krogerus nicht bloss die Käfer sondern wenigstens auch noch die übrigen Arthropoden in seine Untersuchungen einbezogen und in grösserem Ausmass die Fauna gleichgrosser Flächen annähernd quantitativ verglichen hätte. Ebenso hätte sicherlich die Abgrenzung der Tiergesellschaften nach dem Artenbestande, nicht nach Standorten noch klarere Ergebnisse gezeigt.

An die Untersuchungen von Brundin lehnen sich diejenigen von Renkonen (1938) methodisch in vieler Hinsicht an. Auch Renkonen untersucht hinsichtlich ihrer Vegetation möglichst einheitliche Flächen ohne sich an bestimmte Ausmasse derselben zu halten. Um seine Fänge quantitativ miteinander vergleichen zu können, greift er auf die Zeitfangmethode Dahls zurück, auf eine Methode, die nur deshalb bei seinen Untersuchungen brauchbare Vergleichswerte liefert, weil die Zahl der Käferarten und -individuen in den untersuchten nordischen Bruchmoorgebieten verhältnismässig gering und dadurch leicht zu überblicken ist. Hätte Renkonen auch nur wie Krogerus die Gesamtheit der Arthropoden bei seinen Aufnahmsarbeiten berücksichtigt, wäre er bei der Zeitfangmethode schon auf erhebliche Schwierigkeiten gestossen und hätte er ausserdem noch in einem dichter besiedelten Gebiete mit stärker wechselnden Vegetationsverhältnissen gearbeitet, wäre er ohne Untersuchung von Probeflächen bestimmter Grösse überhaupt nicht mehr zu vergleichbaren Resultaten gelangt.¹ Die Gesamtheit der innerhalb einer homogenen Probefläche gefundenen Käferarten nennt Renkonen in Anlehnung an die Terminologie Palmgréns (1928, 1930) "Bestand", im wesentlichen gleichartige Bestände vereinigt er zu Bestandestypen, die in ihrer systematischen Rangordnung etwa den Assoziationen der Pflanzensoziologie entsprechen. Die Verwandtschaft der Bestände kommt im Gleichheitsgrad der Arten- und Individuenzusammensetzung zum Ausdruck, wobei den dominanten Arten für die Bestandescharakteristik besondere Bedeutung beigemessen wird. Renkonen findet, dass innerhalb jeder Probefläche eine oder doch ganz wenige Arten bei seinen Untersuchungen die übrigen erheblich an Individuenzahl übertreffen und er fasst diese dominierenden Arten ähnlich wie die schwedischen Pflanzensoziologen als Charakterarten der Bestandestypen auf. Treuegradbestimmungen hat er nicht durchgeführt, die Konstanz nur schätzungsweise durch Vergleich der verwandten Bestände festgestellt. Er unterscheidet eine ganze Reihe von Bestandestypen, die sich auf vier Verwandtschaftsgruppen verteilen. Renkonen hat seine Probeflächen in Hinblick auf die von Cajander beschriebenen Bruchmoortypen gewählt und abgegrenzt. Er kommt am Schlusse seiner Untersuchungen zu dem Ergebnis, dass die von den terrestrischen Käferarten der Bruchmoore gebildeten Bestandestypen in einer viel höheren Masse von klimatischen Faktoren abhängig sind als die Bruchmoortypen Cajanders selbst, derart dass innerhalb eines Moortypus oft eine grössere Zahl von

¹ Bei der Untersuchung der Mikrofauna des Bodens versagt die Zeitfangmethode vollständig.

Bestandestypen festzustellen ist. Dieses Ergebnis ist zwar nach den an früherer Stelle gemachten Erwägungen durchaus möglich scheint aber doch darauf hinzuweisen, dass entweder die von Cajander beschriebenen Bruchmoortypen noch nicht alle tatsächlich vorhandenen Pflanzengesellschaften einschliesslich der Subassoziationen beinhalten oder aber dass Renkonen infolge der kleinen von ihm berücksichtigten Auswahl aus den vorhandenen Tierarten und der Nichtberücksichtigung des Treuegrades der Bestandescharakterarten mehr Tiergesellschaften beschrieben hat, als tatsächlich vorhanden sind. Ob diese Vermutung richtig ist, werden erst weitere Untersuchungen über den soziologischen Aufbau der Bruchmoore Skandinaviens zeigen.

Der vorstehende Überblick, der wie schon erwähnt, keinerlei Vollständigkeit anstrebt, sondern nur die wichtigsten Arbeiten berücksichtigt, zeigt zur Genüge, wie sehr die tiersoziologische Forschung noch in ihrem Anfängen steht und wie sehr ihr bis heute begriffliche und methodische Einheitlichkeit fehlt. Zudem entbehren auch die ausgereiftesten tiersoziologischen Arbeiten, wie die schönen Studien von Krogerus und Brundin heute einer letzten Konsequenz hinsichtlich der synökologischen Auswertung der erzielten Ergebnisse. Es soll nun in den nächsten Kapiteln untersucht werden, ob es möglich ist, die reicheren Erfahrungen der Pflanzensoziologie in der Tiersoziologie sinngemäss anzuwenden und damit auch diese auf ein festeres Fundament zu stellen.

III. DIE SOZIOLOGISCHE GRUNDEINHEIT

Die Grundvoraussetzung für eine klare und eindeutige Beschreibung von Gesellschaftsindividuen und Gesellschaftstypen in der Biosoziologie ist das Vorhandensein eindeutiger Kriterien für deren Charakteristik und Abgrenzung. Über solche Kriterien verfügt die Pflanzensoziologie heute in ausreichendem Masse.

Wie schon eingangs erwähnt bildet die Assoziation die Grundeinheit in der pflanzensoziologischen Gesellschaftssystematik. Als Assoziationsindividuum¹ wird in der Pflanzensoziologie ein Pflanzenbestand bezeichnet, der in sich homogen ist, das heisst innerhalb seiner Grenzen einheitliche floristische Zusammensetzung und gleiche Relief- und Bodenverhältnisse aufweist (Braun-Blanquet, 1928). Selbstverständlich kann ein solches Assoziationsindividuum je nach der grösseren oder geringeren Einheitlichkeit des Geländes und der Vegetation das eine Mal eine grössere, das andere Mal eine kleinere Fläche umfassen. Der Vergleich der Assoziationsindividuen erfolgt auf Grund ihres gesamten floristischen Aufbaues, der durch die vollständige Artenliste und eine Reihe quantitativer und qualitativer analytischer Gesellschaftsmerkmale gekennzeichnet ist. Als quantitative Merkmale sind Individuenzahl, Deckungsgrad, Häufungsweise (Soziabilität), Frequenz (Gleichmässigkeit der Verteilung), an qualitativen Schichtung, Vitalität und Periodizität des Auftretens zu nennen. Der Assoziationstypus ergibt sich aus dem Vergleiche einer grossen Zahl im wesentlichen gleichartiger Assoziationsindividuen, die aus einem möglichst ausgedehnten Gebiete stammen sollen. Der Assoziations-

¹ Ich folge hier vorwiegend der Auffassung der Schweizer pflanzensoziologischen Schule.

typus, die Assoziation in ihrer abstrakten Bedeutung, ist in erster Linie durch das Vorhandensein für sie kennzeichnender Charakterarten und durch die für sie bezeichnende Artenverbindung charakterisiert. Charakterarten sind nach Aichinger (1933) in Anlehnung an Braun-Blanquet "solche, die ausschliesslich in einer Gesellschaft vorkommen (treue Charakterarten), oder die doch eine bestimmte Gesellschaft bevorzugen, wenn sie auch spärlich in verwandten Assoziationen vorkommen (feste Charakterarten) oder solche Arten, die in mehreren Gesellschaften reichlich vertreten sind, jedoch eine davon bevorzugen und darin ihr optimales Gedeihen finden (holde Charakterarten)". Die Charakterarten sind mit anderen Worten durch ihren hohen Grad von Gesellschaftsstetigkeit (das mehr oder weniger ständige Vorhandensein in den untersuchten Einzelbeständen) und durch ihre ausgeprägte Gesellschaftstreue (Grad der Gesellschaftsgebundenheit) ausgezeichnet.¹ Stetigkeits- und Treuegrad werden auch als synthetische Gesellschaftsmerkmale bezeichnet, weil sie zusammen mit der charakteristischen Artenverbindung zur Charakterisierung des Gesellschaftstypus dienen. Die charakteristische Artenverbindung ist in den Charakterarten in Verbindung mit den Arten höchster Stetigkeitsgrade (Arten die in mehr als 60% der untersuchten Einzelbestände vorkommen) gegeben.

Der Assoziationsbegriff, mit dem die moderne Pflanzensoziologie arbeitet, ist, wie aus dem Vorstehenden wohl deutlich genug hervorgeht, klar auf Grund exakt feststellbarer soziologischer Gegebenheiten definiert. Seine Definition ist eine solche, dass er mit geringfügigen Abänderungen auch in der Tiersoziologie angewendet werden kann und durchaus berufen erscheint, dortselbst den reichlich unklaren Biocönosebegriff zu ersetzen. *Ich schlage daher vor, von nun an auch in der Tiersoziologie ausschliesslich mit der Assoziation als soziologischer Grundeinheit zu arbeiten*, wie dies bereits von Franz-Höfler-Scherf (1937) versucht worden ist. Es ist dabei ohne weiteres möglich, um Unklarheiten zu vermeiden, von Phyto- und Zooassoziationen zu sprechen und den Begriff Assoziation den Gesellschaftsverbindungen von Tieren und Pflanzen vorzubehalten, deren gemeinsame Erfassung mir in Übereinstimmung mit Hesse (1924) als oberstes soziologisches Ziel erscheint.

Es fragt sich nun, ob das gesellschaftliche Gefüge der Tierwelt tatsächlich, gleich dem der höheren Pflanzen, allenthalben einen so gesetzmässigen Aufbau erkennen lässt, dass die Abgrenzung von Zooassoziationen möglich und gerechtfertigt ist. Lässt auch die Tierwelt wie die höhere Vegetation überall gesetzmässig unter gleichen Bedingungen wiederkehrende Artenverbindungen und in hinreichender Zahl für diese kennzeichnende Charakterarten erkennen, so hat diese Frage als im positiven Sinne entschieden zu gelten. Die Aufgabe des folgenden Kapitels wird es sein, das tatsächliche Vorhandensein von Charakterarten und typischen Artenverbänden im Bereiche der Tierwelt nachzuweisen.

¹ Die schwedische pflanzensoziologische Schule misst dem Treuegrad geringere Bedeutung bei, sie setzt an seine Stelle bei Bestimmung der Charakterarten die Dominanz. Der dadurch entstehende Unterschied in der Definition der Charakterarten erscheint im ersten Augenblick erheblicher als er tatsächlich ist, da die dominierenden Arten stets einen hohen Stetigkeitsgrad besitzen und sehr oft auch ausgesprochen gesellschaftstreu sind.

IV. CHARAKTERARTEN UND CHARAKTERISTISCHE
ARTENVERBINDUNGEN

Es ist ein weitverbreitetes Vorurteil, dass Tiere wegen ihres zumeist verhältnismässig grossen Lokomotionsvermögens eine geringe Ortsstetigkeit besitzen. Beobachtungen, die leider erst in geringer Zahl in einzelnen Biotopen über längere Zeiträume hinweg angestellt worden sind, haben gezeigt, dass in unseren Landtiergesellschaften ein ganz beträchtlicher Teil des Artenbestandes durch grosse Ortsgebundenheit ausgezeichnet ist. Ich gebe dafür nur einige Beispiele.

Brunner (1881) und Franz (1933) haben gefunden, dass gewisse über gutes Flugvermögen verfügende Heuschreckenarten in den xerothermen Gebieten Südostmitteleuropas dauernd auf kleine, ja kleinste Reliktstandorte beschränkt leben und ausserhalb dieser nie gefunden werden. Valle wies (1927) nach, dass zwischen der Verbreitung der Orthopteren und dem Vegetationstypus enge Zusammenhänge bestehen. Holdhaus (1911) hat gezeigt, dass die echten Gebirgstiere unter den Arthropoden an festes Gestein gebunden sind und niemals auf junges Anschwemmland übertreten. Jeannel (1926) hat die extreme Anpassung der Höhlenfauna an ihre Umgebung eingehend untersucht und dadurch die äusserst beschränkte Verbreitung der meisten echten Höhlentiere verständlich gemacht.

Ausgesprochene Standortstreue und Gesellschaftsstetigkeit zahlreicher Kleintierarten wird auch durch die Untersuchungen von Krogerus (1932), Brundin (1934) und Renkonen (1938) erwiesen. Das Bedeutsame an den Feststellungen der letztgenannten Autoren in Bezug auf die hier behandelte Frage liegt darin, dass sie für eine grosse Zahl von Assoziationen verschiedenster Art typische Charakterarten nachweisen konnten. Krogerus zeigte, dass am sandigen Meeresstrand, auf fast vegetationslosen Dünenhügeln und auf den bereits durch dichtere Vegetation gefestigten Tribsandflächen jeweils ganz bestimmte Tierarten leben und dass nicht wenige von diesen ausschliesslich oder fast ausschliesslich für einen einzigen der genannten Standortstypen charakteristisch sind. Brundin wieder zeigte, dass in dem an verschiedenen Standortstypen reichen Torneträskgebiete schon bei alleiniger Berücksichtigung der Ordnung der Coleopteren fast jedem Standortstyp seine besonderen Charakterarten zukommen. Wir haben demnach heute bereits aus allen Arten von Landbiocönosen Beweise für das Vorhandensein typischer Zooassoziationen vorliegen. Die wenigen tiersoziologischen Untersuchungen, die zu gegenteiligen Ergebnissen geführt haben, erweisen sich bei kritischer Überprüfung hinsichtlich ihrer Methodik oder hinsichtlich des Umfanges der gemachten Aufnahmen als unzulänglich. So konnte Frenzel (1936), um nur ein Beispiel anzuführen, zweifellos deshalb keine deutlichen Unterschiede in den Tierbeständen der von ihm untersuchten Wiesenböden nachweisen, weil die von ihm quantitativ aufgenommen Probeflächen zu klein (10 mal 10 beziehungsweise 25 mal 25 cm) und zu wenig zahlreich waren. Zudem gehörten anscheinend nicht nur die von ihm untersuchten Wiesen als ganzes botanisch verschiedenen Assoziationstypen an, sondern dieselben scheinen überdies auch in sich nicht einheitlich gewesen zu

sein. Dazu kommt noch, dass man aus vegetationsgeschichtlichen Gründen annehmen muss, dass wohl alle von Frenzel untersuchten Wiesenflächen vor kürzerer oder längerer Zeit noch Wald und zwar Wald von recht verschiedenem Typus waren und aus diesem Grund eine mehr oder weniger veränderte und wohl auch verarmte Fauna aufweisen. Alle diese Umstände wurden von Frenzel nicht gebührend berücksichtigt, obwohl sie von grundlegender Bedeutung für die Erkennung, Abgrenzung und Bewertung der aufgenommenen Tierbestände gewesen wären. Dass planmässige und eingehende Untersuchungen unbedingt auch in der Bodenfauna Unterschiede zutage fördern werden, scheint mir schon heute durch eine Reihe von Beobachtungen erweisbar. Man weiss seit langer Zeit, dass auf und in Böden verschiedener physikalischer und chemischer Beschaffenheit erheblich verschiedene Tiergesellschaften leben. Man spricht danach von psammophilen, petrophilen, halophilen und calciphilen Arten und Gesellschaften. Ebenso weiss man, dass sich in unseren Gebirgen und besonders in denen durch die Eiszeit nicht devastierter südlicher Länder eine Bodenfauna von grossem Artenreichtum findet, die je nach dem Standortstypus eine sehr verschiedene Artenzusammensetzung besitzt. Von einer weitgehenden Übereinstimmung der Bodenfauna in Wäldern und Wiesen oder auch im Gebirge und in der Ebene, wie sie Frenzels Untersuchungen anzuzeigen scheinen, kann in Wirklichkeit besonders bei Berücksichtigung ungestörter Assoziationen keine Rede sein. Man kann darum schon heute sagen, dass sich allenthalben in der Zusammensetzung der Landtierwelt Tiergesellschaften mit für sie charakteristischen Artenverbindungen und Charakterarten feststellen lassen.

Aber nicht nur in der Land- sondern auch schon in der Wasserfauna, besonders der Bodenfauna der Binnengewässer und der Fauna der Meeresküsten zeichnen sich auf Grund neuerer Untersuchungen immer deutlicher gut charakterisierbare Assoziationen ab. Wir wollen hier auf die Tiergesellschaften der Meeresküsten nicht weiter eingehen, obwohl gerade hierüber seit der klassischen Arbeit von Meyer und Moebius über die Fauna der Kieler Bucht bereits eine Reihe bedeutsamer Untersuchungen vorliegen, sondern uns im folgenden mit einigen kurzen Hinweisen auf die mit der Landtierwelt in engerer Verbindung stehenden Süsswasserassoziationen begnügen. Dass Thienemann eine strenge Bindung gewisser Fliegenlarven an bestimmte Seentypen festgestellt hat, wurde schon an früherer Stelle besprochen. Durch Thienemanns Entdeckung angeregt haben andere Forscher wie Lundbeck (1926), Valle (1927, 1928), und Decksbach (1929) die Bodenfauna der Binnenseen genauer studiert und weitere Zusammenhänge zwischen Faunenbestand und Umwelt aufgedeckt. Mit besonderer Deutlichkeit kommt die reiche Gliederung der Süsswasserfauna in der Arbeit von Ekman (1915) über die Bodenfauna des Vättern zum Ausdruck. Obgleich die genannten Untersuchungen in soziologischer Hinsicht sicherlich noch weit von ihrem Abschluss entfernt sind, ist doch festzustellen, dass wir schon heute auch innerhalb der Bodenfauna unserer Binnenseen streng stenotope Arten kennen, die aller Wahrscheinlichkeit nach gute Charakterarten noch genauer zu umgrenzender Zooassoziationen darstellen. Eine recht auffällige Bindung an bestimmte Boden- beziehungsweise Strömungsverhältnisse

ist auch bei zahlreichen Arten der Bodenfauna unserer Bäche und Flüsse festzustellen. Auch hier werden sich verschiedene Tierassoziationen auf Grund guter Charakterarten unschwer gegeneinander abgrenzen lassen. Leitformen der extremsten Assoziationen der Gletscherwässer hat Steinböck (1934) angegeben, einen meines Wissens ersten Versuch die Torrenticolfauna hinsichtlich ihrer quantitativen Zusammensetzung zu studieren hat Geijskes (1935) im Baseler Tafeljura unternommen. Die letztgenannte Arbeit gibt zwar keine ausgesprochenen Assoziationsbeschreibungen, lässt aber doch auf Grund quantitativer Aufnahmen erkennen, dass die Fauna des untersuchten Baches an verschiedenen Stellen erhebliche Unterschiede sowohl hinsichtlich des Arten- als auch hinsichtlich des Individuenbestandes aufweist. Auch eine Arbeit von Beyer (1932) über die Tierwelt der Quellen und Bäche des Baumberggebietes gestattet einen gewissen Einblick in die gesellschaftliche Struktur der Süßwassertierwelt.

Die vorstehenden Ausführungen haben wohl schon hinlänglich nachgewiesen, dass sich auch in der Tierwelt ebenso wie in der Pflanzenwelt überall scharf durch Charakterarten und durch eine bestimmte Artenverbindung gekennzeichnete Assoziationen feststellen lassen. Es seien jedoch trotzdem nachfolgend noch einige ausführlichere Beispiele aus eigenen tiersoziologischen Aufnahmen angeführt, weil sich an diesen der hohe Grad von Gesellschaftstreue und -stetigkeit der tierischen Leitformen noch deutlicher aufzeigen lässt.

Auf Salzböden mit starker Salzanreicherung (pH um 9.0 und vermutlich allgemein starkem Na_2CO_3 -Gehalt des Bodens) und nicht zu geringer Bodenfeuchtigkeit treten gemeinsam mit einer äusserst dürrtigen Vegetation extremer Salzpflanzen, vor allem *Suaeda maritima* Dum., im Salzlachengebiet des Neusiedlerseebeckens, an den Salzstellen in der Grossen Ungarischen Tiefebene und vermutlich weit darüber hinaus einige extreme Salzkäfer auf. Zu den ausschliesslich an derart extremen Standorten lebenden Kleintieren gehören die Käferarten *Pogonus luridipennis* Germ., *Bembidion ephippium* Marsh., *Dyschirius pusillus* Dej. und *Bledius unicornis* Germ. Die genannten Arten sind durchwegs gesellschaftstreue Charakterarten einer Zooassoziation extremer Salzflächen die allerdings durch eingehende tiersoziologische Untersuchungen noch genauer charakterisiert werden muss. In ähnlicher Weise scheinen auch die anderen streng an Salzböden gebundenen oder doch solche bevorzugenden Insektenarten gesellschaftstreue oder doch gesellschaftsfeste Charakterarten verschiedener noch näher zu beschreibender und gegeneinander abzugrenzender Salzassoziationen zu sein (vgl. Franz-Höfler-Scherf, 1937).

Auf extrem xerothermen Steppenböden, die dauernd waldfrei waren und abgesehen von vorübergehender Beweidung keine einschneidenden künstlichen Veränderungen erlitten haben, findet sich in den Trockengebieten östlich von Wien, in den südlichen Teilen der Slowakei, im ungarischen Mittelgebirge und wohl noch weiter gegen Süden und Südosten darüber hinaus eine Wärme und Trockenheit liebende Tiergesellschaft, der eine ganze Anzahl von Charakterarten eigentümlich sind. Zu den gesellschaftstreuen Charakterarten dieser Assoziation gehören nach den Untersuchungen von Franz (1936, 1937) die Käferarten *Harpalus*

saxicola Dej., *Cymindis variolosa* F., *C. scapularis* Schaum, *Saprinus amoenus* Redtb., und *Otiorrhynchus mandibularis* Redtb., sowie die Heuschrecke *Celes variabilis* Pall. Als feste Charakterarten sind ausserdem noch anzusprechen die Käferarten *Chrysomela cerealis* var. *alternans* Panz. und *Chr. limbata* F., die Heuschrecken *Stenobothrus nigromaculatus* Herr. Sch. und *Omocestus petraeus* Bris., sowie die Wanze *Coranus subapterus* D.G. Unter den holden Charakterarten dieser Tiergesellschaft verdienen besonders die Käferarten *Harpalus anxius* Duft. und *Pedinus femoralis* L., sowie die Wanze *Sciocoris cursitans* L. Erwähnung. Von grösseren Tieren ist der mitteleuropäische Ziesel *Citellus citellus* L. als holde Charakterart dieser Assoziation zu werten, wenn er auch infolge seiner bedeutenderen Körpergrösse naturgemäss in wesentlich geringerer Individuenzahl auftritt, als die meisten der angeführten Arthropoden. Diese gehören fast durchwegs dem Sommeraspekt der Assoziation an und sind im Frühling und Spätherbst nicht zu finden. Sie sind dann zum Teil durch andere Insektenarten ersetzt von denen der Laufkäfer *Oodes gracilis* Villa als holde Charakterart der Assoziation besondere Erwähnung verdient.

Einen überaus grossen Reichtum an zumeist sehr gut charakterisierten Tiergesellschaften weist die Tierwelt des Hochgebirges auf. Auch hier ist die Ermittlung der Charakterarten verhältnismässig einfach. Ich führe nachfolgend nur zwei Beispiele von Zooassoziationen aus noch nicht veröffentlichten eigenen soziologischen Untersuchungen an, die ich in den beiden letzten Jahren mit besonderer Förderung des Deutschen Alpenvereines im Gebiete des Grossglockners in den Hohen Tauern anstellte.¹

Unter den hochalpinen Tiergesellschaften dieses Gebietes fallen einige besonders auf. Eine der auffälligsten ist eine zwar artenarme aber scharf umrissene Assoziation, die regelmässig auf sandig verwitternden, sonnigen Kalkphyllithalden anzutreffen ist. Eine absolut gesellschaftstreue Charakterart dieser Assoziation ist die Milbe *Caeculus echinipes* Dufour (C. Willmann), ein Tier, das sich durch einen sehr hohen Grad von Gesellschaftstetigkeit auszeichnet. Ebenso gesellschaftstreu aber als extremes Relikt weniger gesellschaftstet ist der Blattkäfer *Chrysomela crassicornis* subsp. *norica* Holdh., der nur in klimatisch besonders begünstigten, hochgelegenen Assoziationsindividuen des beschriebenen Assoziationstypus vorkommt. Als holde Charakterart der Gesellschaft ist ferner der Rüsselkäfer *Otiorrhynchus chalcus* Strl. zu nennen. Die Vegetation deckt in der beschriebenen Assoziation den aus sandigem Schutt bestehenden Rohboden, der noch keine Humusdecke trägt, nur zum geringen Teil. Die Vegetation besteht vorwiegend aus *Linaria alpina* Miel., *Saxifraga oppositifolia* L., *Taraxacum alpinum* Hgtsch. et Heer und *Silene acaulis* L., zu denen sich an etwas vegetationsreicheren Stellen häufig *Dryas octopetala* L. und kümmerliche Gräser gesellen. Die Assoziation ist eine ausgesprochene Pioniergesellschaft.

Eine zweite sehr charakteristische Assoziation findet sich im Glocknergebiet in rauhen, schneereichen Lagen in grosser Höhe (nie unter 2500 m.). Dieser Assoziation gehört als absolut gesellschaftstreu Charakterart von hohem Stetig-

¹ In den nachfolgend mitgeteilten Ergebnissen ist die Mikrofauna des Bodens noch nicht mit berücksichtigt.

keitsgrad der Laufkäfer *Nebria atrata* Dej. an, als gesellschaftsfeste Charakterart ist ihr der Schmetterling *Gnophos caelibarius intermedius* Kautz zuzurechnen. Die Bestimmung einiger Milben, welche die Assoziation als holde Charakterarten kennzeichnen, steht derzeit noch aus. In der den Boden nur lückenhaft deckenden Vegetation tritt meist *Saxifraga Rudolphiana* Hornsch. beherrschend auf.

Die angegebenen Beispiele könnten leicht beliebig vermehrt werden. Es gibt nicht nur in der hochalpinen Zone im Bereiche der hochgelegenen Grasmatten, der Schneetälchen, Moränenhalden und sumpfigen Rieder allenthalben weitere charakteristische Tiergesellschaften, sondern es finden sich solche auch in den subalpinen Wäldern und im Sumpfland der Ebene in sehr ausgeprägter Form.

Am schwierigsten wird sich vermutlich die Erfassung der Tierwelt des Waldlandes, der Wiesen und des Ackerlandes gestalten. Dies sind Biotope, die einerseits eine sehr grosse Zahl von Tierarten beherbergen und anderseits zumeist auch in ihrem Tierbestand durch die Kulturtätigkeit des Menschen erhebliche und oft nur schwer feststellbare Veränderungen erfahren haben. Es dürfte sich empfehlen, die Analyse besonders komplexer Tierverbände erst dann in Angriff zu nehmen, wenn die übersichtlicheren Tiergesellschaften des Gebietes einigermaßen erforscht sind. Es werden sich dann die artenreichsten, unübersichtlichsten Verbände bei fortschreitender Erforschung der in ihrer Nachbarschaft vorkommenden einfacheren Assoziationen allmählich von selbst umgrenzen und charakterisieren.

Jedenfalls lassen die angeführten Beispiele keinen Zweifel darüber entstehen, dass auch innerhalb der Tierwelt klare synökologische Gesetzmässigkeiten bestehen, und man muss nur darüber staunen, dass die so auffälligen tiersoziologischen Zusammenhänge nicht schon bisher in höherem Masse zu ihrer Erforschung Anlass gegeben haben. Es scheint mir, dass die Ursache dieses Versäumnisses weniger darin zu suchen ist, dass die synökologischen Phänomene übersehen wurden, als vielmehr darin, dass es an geeigneten Methoden zu ihrer Untersuchung gebrach. In dem folgenden Schlusskapitel soll versucht werden, in Anlehnung an die Pflanzensoziologie auch in der Tiersoziologie methodisch exaktere Wege zu beschreiten.

V. ZUR METHODIK TIERSOZIOLOGISCHER AUFNAHMEN

Es wurde schon oftmals in der tiersoziologischen Literatur darauf hingewiesen, dass die Erforschung der Synökologie der Tiere mit ungleich grösseren Schwierigkeiten verbunden ist, als diejenige der Pflanzengesellschaften. Die grösste Schwierigkeit bei tiersoziologischen Aufnahmen besteht wohl darin, dass es unmöglich ist, ähnlich wie bei Vegetationsaufnahmen die Zusammensetzung des Tierbestandes einer bestimmten Lokalität schon bei einer flüchtigen Begehung annähernd zu überblicken, sondern dass es hierzu mühsamer und zeitraubender Aufsammlungen der Kleintierwelt bedarf.

Dies ist auch der Grund, weshalb tiersoziologische Untersuchungen immer von den Standortstypen und Vegetationseinheiten ausgehen müssen. Sie dürfen aber nicht bei diesen stehen bleiben, sondern müssen in jedem einzelnen Falle klarstellen,

ob sich die Grenzen einheitlicher Pflanzen- und Tierbestände decken, wie sie sich zu den Standortverhältnissen verhalten und falls Unterschiede in der Verhaltensweise bestehen, worauf dieselben zurückzuführen sind.

Um die genannten Fragen beantworten zu können, bedarf es exakter Aufnahmen der einzelnen Tierbestände, zu deren Erlangung quantitative Untersuchungen unbedingt erforderlich sind. Über die zur quantitativen Erfassung von Kleintierbeständen anzuwendenden Methoden ist mehr geschrieben worden als von Nutzen war. Ich möchte es daher vermeiden, die an sich wenig nutzbringende Literatur auf diesem Gebiete durch weitere langwierige Ausführungen zu vermehren. Es sei darum nur in aller Kürze festgestellt, dass nach meinen Erfahrungen mit der vielfach angewandten Zeitfangmethode mangels genügender Objektivität und wegen der Verschiedenheit der Sammelverhältnisse in den einzelnen Biotopen keine befriedigenden Ergebnisse erzielt werden können. Die einzige wirklich exakte Sammelmethode ist die möglichst vollständige Erfassung aller in einheitlichen Flächenquadraten bestimmter Grösse vorhandenen Tiere, was in der Weise zu geschehen hat, dass man stets von den Rändern zur Mitte des Quadrates fortschreitend, zunächst die Kraut- und danach die Bodenschicht der Probefläche untersucht. Hierbei leisten Kätscher und Insektensieb die wertvollsten Dienste; das Aussuchen des Gesiebes muss unbedingt mit Hilfe eines Gesiebeautomaten erfolgen. Zur Erfassung der Bewohner von Bäumen und Sträuchern, sowie der grösseren Tierarten sind selbstverständlich andere Methoden anzuwenden.

Hinsichtlich der Frage, wie gross die Probeflächen bei Untersuchung der Kraut- und Bodenschicht nach Kleintieren gewählt werden müssen, besteht heute noch keine Einigkeit. Nach meinen Erfahrungen müssen zur Untersuchung der Arthropodenfauna der Boden- und Vegetationsschicht je nach den Verhältnissen Flächen von 1 bis 4 m² untersucht werden. Beschränkt man die Aufsammlungen auf Flächenquadrate geringeren Ausmasses, so erfasst man mehr oder minder unvollständige Artenbestände, deren statistische Auswertung kein klares Bild über die Zusammensetzung der Gesamtassoziation liefert. Flächen von 400 cm² oder gar nur 100 cm² sind unter allen Umständen zu klein. Es ist mir klar, dass die annähernd quantitative Erfassung der auf Flächen von 4 m² lebenden Kleintierbestände erhebliche Mühe und Zeit kostet, aber ich sehe derzeit keinen anderen Weg, um wirklich exakte Aufnahmen zu machen. Etwas vereinfacht wird die Arbeitsweise dadurch, dass es genügt, *annähernd* quantitative Aufsammlungen durchzuführen. *Absolut* quantitative Werte haben nur für die Untersuchung produktionsbiologischer Fragen Bedeutung.

Für die Untersuchung der Tiergesellschaften genügt es, jeweils annähernd alle vorhandenen Arten und deren ungefähre Individuenzahl zu ermitteln. Dass die äusserst mühsame Aufsammlung der Mikrofauna des Bodens (Nematoden, Acarinen etc.) nur in wesentlich kleineren Proben vorgenommen werden kann, ist klar. Es fehlen leider bisher noch Versuche, die eindeutig darüber Aufschluss geben würden, wieviel Bodenfläche untersucht werden muss, um ein einigermaßen vollständiges Bild von den im Boden lebenden Beständen kleinster Metazoen zu liefern. Für die Untersuchung der auf Bäumen und Sträuchern lebenden Tiere dürfte sich die annähernd quantitative Ermittlung der auf einzelnen Baumindividuen lebenden

Bestände unter Vergleich einer grösseren Zahl von Resultaten als der gangbarste Weg erweisen. Die Aufsammlungen werden in diesem Falle, soweit sie Kleintiere betreffen, mit Hilfe des Klopfschirmes oder grosser Klopftücher durchgeführt werden müssen, wobei das Abfliegen einzelner flüchtiger Baumbewohner nicht wird vermieden werden können. Über die quantitative Aufsammlung der im Holz lebenden Kleintierwelt liegen noch keine Erfahrungen vor.

Für die Berücksichtigung grösserer Tiere bei soziologischen Aufnahmen sind ähnlich, wie dies die Pflanzensoziologie bei der Aufnahme von Waldbeständen durchführt und wie dies auch Palmgrén (1930) bei seiner soziologischen Aufnahmen der finnländischen Vogelwelt versucht hat, grössere Flächen zu verwenden. Im Falle der Vogelfauna dürften Flächenquadrate von 100 mal 100 m ausreichend sein. Ob und in welcher Form die so ermittelten Grosstierbestände mit den Kleintiergesellschaften in unmittelbare Beziehung gebracht werden können, wird erst auf Grund eines umfangreicheren Aufnahmsmaterials festgestellt werden können.

Für die Abgrenzung einheitlicher Tierbestände ist die Ermittlung gewisser besonders auffälliger Charakterarten Voraussetzung. Die Feststellung dieser Leitformen erfolgt am besten in der Weise, dass man zunächst innerhalb eines sowohl hinsichtlich seiner Vegetation als auch hinsichtlich der klimatischen und edaphischen Faktoren möglichst einheitlichen Standortes mehrere Probeflächen aufnimmt. Hat man eine grössere Zahl von Quadratuntersuchungen aus einem derartigen Bestande zur Verfügung, so kann man durch Vergleichung der Zusammensetzung der einzelnen gemachten Tierfänge ohne weiteres feststellen, ob der Bestand auch tiersoziologisch einheitlich ist oder nicht. Die Aufarbeitung und der Vergleich der Proben wird allerdings nicht an Ort und Stelle sondern im Laboratorium vor sich gehen müssen. Auf Grund der erzielten Laboratoriumsuntersuchungen hat dann eine zweite Feldaufnahme stattzufinden. Hat die Aufarbeitung der Proben wesentliche Unterschiede in ihrer Zusammensetzung ergeben, so werden die Ursachen dieser Verschiedenheit bei der zweiten Felduntersuchung meist unschwer festgestellt werden können. Dies um so leichter, wenn man bei der ersten Aufnahme die einzelnen Probequadrate gleichsinnig mit den Proben nummeriert und mit Nummernstäbchen versehen hat. Sind einmal auch tiersoziologisch einheitliche Bestände ermittelt, so wird man unschwer eine Anzahl solcher Tierarten finden, die in dem Bestandestypus mit grosser Konstanz auftreten ohne in gleicher Weise auch anderen Tiergesellschaften eigen zu sein. Eine gründliche Kenntnis der gesamten Fauna des Gebietes wird diese Feststellung sehr erleichtern. Ist man so weit gelangt, so kann man daran gehen, weitere gleich oder ähnlich aussehende Bestände zu untersuchen und hinsichtlich ihrer Faunenzusammensetzung miteinander und mit benachbarten anders aussehenden Beständen zu vergleichen. Dabei wird man auf die Beobachtung des Verhaltens der durch ihr charakteristisches Auftreten innerhalb der zuerst untersuchten Tiergesellschaften aufgefallenen Tierformen besonderes Gewicht legen. Es wird so gewöhnlich möglich sein, für die Assoziation infolge ihrer Stetigkeit kennzeichnende Arten zu ermitteln, deren Gesellschaftstreue dann allerdings noch auf Grund vergleichender Untersuchungen in einem grösseren Faunengebiet endgültig festgestellt werden

muss. Zumeist (besonders rasch in engumgrenzten Tiergesellschaften extremer Standorte) gelingt die Ermittlung der Arten hoher Stetigkeits- und Treuegrade, also der Charakterarten schon auf Grund verhältnismässig weniger Exkursionen und es ist dann möglich, die Grenzen der einheitlichen Tierbestände (Gesellschafts-individuen) durch Kartierung der Grenzen der Charakterarten festzustellen. Das geht in allen Fällen viel rascher als die Aufnahme des gesamten vorhandenen Artenbestandes und dient dazu, die Deckung oder Nichtdeckung der Grenzen einheitlicher Tierbestände mit denen der Pflanzengesellschaften und Standortstypen zu ermitteln.

Die Beschreibung des Assoziationstypus muss in der Tiersoziologie wie in der Pflanzensoziologie auf Grund zahlreicher Einzelaufnahmen aus einem möglichst grossen Teilgebiete des Verbreitungsareales der Assoziation erfolgen. Um als Unterlage für die Assoziationsbeschreibung dienen zu können, müssen die Einzelaufnahmen mit grosser Sorgfalt und nach einem im wesentlichen gleichen Grundschema durchgeführt werden. Für die Aufnahme von Landtiergesellschaften kommt etwa folgendes in Anlehnung an die Braun-Blanquetsche Schule entworfene Schema in Betracht.

Es werden von jedem untersuchten Assoziationsindividuum angegeben:

1. Genaue geographische Lage (auch Meereshöhe).
2. Ungefähre Grösse und Umgrenzung des Assoziationsindividuums (Bestandes).
3. Notizen über benachbarte Tiergesellschaften.
4. Beschreibung des Standortes (Grossklima und Standortsklima, Relief, Untergrund und Bodenbeschaffenheit, Vegetation möglichst mit kurzer soziologischer Charakteristik).
5. Zahl und Grösse der quantitativ untersuchten Probestflächen.
6. Datum und Tageszeit der Vornahme jeder einzelnen quantitativen Aufnahme.
7. Analyse des Tierbestandes (Vollständige Artenliste und Abundanz der einzelnen Arten).

Ein ähnliches Schema hat auch schon Oekland (1929) in Vorschlag gebracht.

Die Abundanz (Individuenzahl pro Flächeneinheit) wird am besten mittels einer Abundanzskala ausgedrückt. Franz-Höfler-Scherf (1937) haben in Anlehnung an Braun-Blanquet bei Aufnahme von Insekten- und Spinnenbeständen folgende auf quantitative Aufnahmen von Flächen im Ausmasse von 4 m^2 bezogene Abundanzskala verwendet: $+$ = 1 Individuum der Art in der Probestfläche (die Art ist aber dauernder Bestandteil der Assoziation), $1 = 2$ bis 4 Individuen derselben Art in der Probestfläche, $2 = 5$ bis 10 Individuen, $3 = 11$ bis 25 Individuen, $4 =$ mehr als 25 Individuen (Massenvorkommen). Die Arten, die in die Stufen 3 und 4 fallen, können als dominant bezeichnet werden. Mit einer kleinen hochgestellten Null werden Arten bezeichnet, die zufällig in der Probestfläche vorgefunden wurden, ohne regelmässig in der Assoziation vorzukommen. Die Anwendung dieser Skala bei umfangreicheren soziologischen Aufnahmen wird noch zeigen müssen, ob die getroffenen Stufeneinteilung den praktischen Bedürfnissen entspricht oder ob eine andere Abgrenzung der

einzelnen Stufen vorteilhafter wäre. Wurden innerhalb eines Assoziationsindividuums mehrere Probeflächen untersucht, so können die Ergebnisse in einer Tabelle etwa in folgender Weise zusammengestellt werden.

Speziesname	Individuenzahl in der Probefläche No.				Abundanzzahl
	I	II	III	IV	

Als Abundanzzahl ist der Mittelwert aus den einzelnen Proben einzusetzen. Ausser der Abundanz sind bei den einzelnen Tierarten ähnlich wie bei pflanzensoziologischen Aufnahmen noch folgende Merkmale (analytische Gesellschaftsmerkmale) festzustellen: Häufungsweise oder Soziabilität, Gesamtverteilung oder Frequenz, Schichtung, Vitalität und Periodizität des Auftretens. Bei allen diesen Termini ist die in der Pflanzensoziologie gebräuchliche Definition (vgl. Braun-Blanquet, 1928) ohne weiteres auch in der Tiersoziologie verwendbar. Nur Bestimmungen des Deckungsgrades sind im Bereiche der Tiergesellschaften unmöglich; an ihre Stelle werden vielleicht Angaben darüber treten müssen, ob die Besatzdichte im Vergleich zu Durchschnittsverhältnissen für die betreffenden Arten normal, beziehungsweise unter- oder übernormal ist. Ob sich solche in exakter Weise geben lassen, wird erst durch praktische Versuche ermittelt werden müssen.

Für die Kennzeichnung der Assoziationstypen muss, um dies nochmals hier in diesem methodischen Kapitel zu wiederholen, auch im Bereiche der Tiersoziologie die Feststellung der Charakterarten zusammen mit der charakteristischen Artenverbindung massgebend sein. Es ist notwendig in diesem Zusammenhang noch etwas über die Bestimmung der Charakterarten und der charakteristischen Artenverbindung zu sagen.

Schon im Kapitel III ist darauf hingewiesen worden, dass auch in der Pflanzensoziologie hinsichtlich der Kriterien, auf Grund welcher die Charakterarten zu bestimmen sind, noch keine vollständige Einigkeit besteht. Die schweizer Schule wertet die Charakterarten nach ihrem Stetigkeits- und Treuegrad, während die schwedische Schule den letzteren vernachlässigt und dafür dem Dominanzgrad grosse Bedeutung beimisst. Mir scheinen alle drei Momente für die Kennzeichnung der einzelnen Assoziationstypen wichtig zu sein, wobei aber doch meines Erachtens dem Treuegrad vor der Dominanz der Vorrang eingeräumt werden muss. Ist eine Art in einer Assoziation dominant vertreten, aber auch in anderen Assoziationen stets oder oft vorhanden, so kann sie nicht als Assoziationscharakterart angesprochen werden, muss vielmehr bei der Kennzeichnung der Assoziation an zweiter Stelle (unter den gesellschaftsteten Arten) angeführt werden. Dessen ungeachtet darf der Dominanzfaktor bei Kennzeichnung der Assoziationstypen nicht vernachlässigt werden. Gerade in Tiergesellschaften extremer Standorte dominieren oft einzelne Arten in ihrer Individuenzahl weit über die anderen mit ihnen zusammen vorkommenden, und das ist eine für die betreffenden Assoziationen dann durchaus charakteristische Erscheinung. *Ich schlage daher vor, als synthetische Gesellschaftsmerkmale jeweils auch die Dominanzverhältnisse anzuführen.*

Die charakteristische Artenverbindung wird durch die Arten hoher Stetigkeitsgrade dargestellt. Die von Braun-Blanquet (1928) angegebene Stetigkeitsskala ist

ohne weiteres auch auf tiersoziologischem Gebiete anwendbar. Nach ihr bedeuten: I = die Art ist in 1–20% der Einzelbestände vorhanden, II = in 20–40%, III = in 40–60%, IV = in 60–80%, und V = in 80–100%.

Hinsichtlich des Treuegrades werden auf pflanzensoziologischem Gebiete drei Kategorien von Arten innerhalb der Assoziationen unterschieden, die Charakterarten, Begleiter und zufälligen Einsprenglinge. Die Unterteilung der Charakterarten in treue, feste und holde und die Anwendbarkeit dieser Klassifikation in der Tiersoziologie wurde bereits in den Kapiteln III und IV erörtert, sodass wir uns hier auf die Abgrenzung der beiden restlichen Kategorien beschränken können. Die Begleiter definiert Braun-Blanquet als Arten ohne ausgesprochenen Gesellschaftsanschluss, die zufälligen Arten als solche, die als zufällige Einsprenglinge oder als Relikte früher dagewesener Gesellschaften aufzufassen sind. Charakterarten, Begleiter und zufällige Arten decken sich in ihrer Bedeutung ungefähr mit den Kategorien eucön, tyhocön und xenocön, die Hesse für tiersoziologische Zwecke geschaffen hat; allerdings ist die Grenze der xenocönen Arten sensu Hesse eine noch etwas engere als die der zufälligen Arten sensu Braun-Blanquet.

In jeder Assoziation müssen die jahreszeitlichen Aspekte genau untersucht und in der Assoziationsbeschreibung selbstverständlich miterfasst werden.

Es ist klar, dass sich auch die Aufgaben der Tiersoziologie nicht in der Beschreibung der Gesellschaftstypen erschöpfen. Diese Beschreibung und Abgrenzung bildet vielmehr auch in der Erforschung der Tiergesellschaften nur eine erste Etappe einer weitergehenden Analyse der bestehenden synökologischen Gesetzmässigkeiten. Sie bildet die Voraussetzung für das Studium des Gesellschaftshaushaltes, der Wechselwirkungen zwischen den Tieren untereinander, den Tieren und Pflanzen und nicht zuletzt auch den Einflüssen welchen beide durch die unbelebte Natur ausgesetzt sind. Sie bildet auch die Grundlage für die Erforschung der Gesellschaftsentwicklung, beziehungsweise der Gesellschaftssukzessionen, denen besonders von Seiten der amerikanischen tiersoziologischen Schule schon bisher viel Beachtung geschenkt wurde. Ein wichtiges, noch kaum bearbeitetes Kapitel der synökologischen Forschung bildet die Untersuchung der Frage, in welcher Weise der Mensch verändernd auf die einzelnen Tiergesellschaften eingewirkt hat und noch heute einwirkt. Das Forschungsgebiet der Tiersoziologie ist damit ein ähnliches und ebenso umfangreiches wie das der Pflanzensoziologie. Die zu leistende Arbeit ist infolge der bedeutend grösseren Formenmannigfaltigkeit der Tierwelt im Vergleiche zur Pflanzenwelt und der schon an früheren Stelle mehrfach erwähnten methodischen Schwierigkeiten zweifellos noch umfangreicher. Im Vergleich zu ihr sind die heute zu ihrer Bewältigung zur Verfügung stehenden Kräfte vollkommen unzulänglich. Es wäre dringend zu wünschen, dass ihre Zahl bald erheblich anwachsen möchte.

Schon auf methodischem Gebiete mussten im Vorstehenden viele Fragen, die nicht theoretisch gelöst werden können, sondern zu ihrer Lösung der praktischen Erfahrung und eigens zu diesem Zwecke angestellter Versuche bedürfen, offen gelassen werden. Ihre Bearbeitung wie die Durchführung umfassender tiersoziologischer Studien überhaupt ist einem einzelnen Forscher kaum möglich. Es ist

daher höchste Zeit, dass sich auch Institute und Arbeitsgemeinschaften die Bearbeitung tiersoziologischer Fragen zur Aufgabe machen, denn nur dann kann der Vielfalt der auf tiersoziologischem Gebiete zu leistenden Arbeit einigermaßen entsprochen werden.

VI. ZUSAMMENFASSUNG

Die vorliegende Untersuchung dient der Klärung terminologischer und methodischer Fragen der Tiersoziologie. Sie berücksichtigt hauptsächlich die Kleintierbestände der Landtiergesellschaften.

In einer Übersicht über die einschlägige Literatur wird nachgewiesen, dass die Tiersoziologie bisher einer einheitlichen Terminologie und klarer Ziele entbehrt. Die Biocönose erweist sich als Grundeinheit für die Einteilung und Klassifikation der Tiergesellschaften als unbrauchbar. Sie ist von den verschiedenen Autoren sehr verschieden definiert worden, jedoch niemals so, dass auf Grund ihrer Definition die Abgrenzung von Gesellschaftsindividuen und Gesellschaftstypen in der Natur mit annähernd gleicher Exaktheit durchgeführt werden könnte wie auf Grund des Assoziationsbegriffes der Botanik.

Es wird daher vorgeschlagen, von nun an auch in der Tiersoziologie von der Assoziation als soziologischem Einheitsbegriff auszugehen und die Assoziation wie in der Pflanzensoziologie durch ihre Charakterarten und ihre charakteristische Artenverbindung zu kennzeichnen. Der Klarheit der Terminologie halber sind die pflanzlichen Gesellschaftseinheiten als Phytoassoziationen, die tierischen als Zooassoziationen zu bezeichnen. Der Terminus "Assoziation" bleibe den Lebensgemeinschaften von Pflanzen und Tieren vorbehalten.

An einer Reihe von Beispielen wird nachgewiesen, dass es auch in den Tiergesellschaften allenthalben Charakterarten gibt, die sich durch einen hohen Grad von Gesellschaftstreue und Gesellschaftstetigkeit auszeichnen; es ist daher auch im Bereiche der Tierwelt überall möglich, Assoziationen zu erkennen und gegeneinander abzugrenzen.

Für die tiersoziologische Aufnahmepaxis und die Beschreibung der Assoziationen werden in enger Anlehnung an die Pflanzensoziologie Vorschläge erstattet, nachdem untersucht worden ist, inwieweit die pflanzensoziologischen Untersuchungsmethoden auch in der Tiersoziologie Anwendung finden können. Die Bestandesanalyse hat auf tiersoziologischem Gebiete durch annähernd quantitative Aufnahme der in einheitlichen Probeflächen von gleicher Grösse vorhandenen Tiere zu geschehen. Für die Ermittlung des Arthropodenbestandes sind Flächen von mindestens 1 m² notwendig.

Für die Charakterisierung der Assoziationstypen ist in der Tiersoziologie wie in der Pflanzensoziologie die Ermittlung der Charakterarten und der charakteristischen Artenverbindung erforderlich. Von Charakterarten ist in erster Linie ein hoher Grad von Gesellschaftstreue und Gesellschaftstetigkeit zu fordern, das dominante Auftreten einer Charakterart in der zu kennzeichnenden Assoziation ist aber jeweils mit zu berücksichtigen.

VII. SUMMARY

The present study deals with questions of terminology and method in animal ecology, particularly regarding communities of smaller land animals.

From a consideration of the literature it is evident that up to the present animal ecology has lacked both a uniform terminology and clear aims. The concept of biocoenosis turns out to be useless as a unit for the subdivision and classification of animal communities. It has been defined in quite different fashions by different authors, and never in such a way that it could be used, as the association concept is used in botany, for the exact delimitation of individual animal communities and types of animal community in nature.

It is therefore now proposed to make use of the association as the sociological unit in animal as well as plant ecology, and to define the association, as is done in botany, by its characteristic species and groups of species. For the sake of clarity, plant ecological units should be termed phytoassociations, animal units zooassociations, the word "association" being reserved for groups of both animals and plants.

By a series of examples it is shown that characteristic species with a high degree of ecological stability are to be found everywhere in animal just as in plant communities; it is therefore possible to recognize associations everywhere in the animal world, and to delimit them from one another.

After an investigation of the extent to which the methods of plant ecology are applicable to animal communities, methods closely following botanical practice are proposed for the purpose of making animal ecological surveys and for the description of associations. The analysis of populations in animal ecology should be carried out by an approximately quantitative census of the animals present in sample areas of equal dimensions.

In defining types of associations, the determination of characteristic species and groups of species is essential in animal ecology just as in plant ecology. A characteristic species must exhibit a high degree of ecological constancy. The dominant presence of a characteristic species is likewise of importance.

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NACHTRAG

Während der Drucklegung der vorliegenden Arbeit wurden vom Verfasser zusammen mit M. Beier, W. Rouschal und Chr. Wimmer umfangreiche tiersoziologische Untersuchungen von Wiesen- und Ackerböden in Angriff genommen. Bei diesen haben sich die hier vorgeschlagenen Aufnahmsmethoden bestens bewährt. Einer gewissen Ergänzung bedürfen die gemachten methodischen Vorschläge nur hinsichtlich der zur Charakteristik der Tiergesellschaften empfohlenen Abundanzskala.

Es hat sich nämlich herausgestellt, dass die in Flächen bestimmter Grösse vorhandenen Individuenzahlen der einzelnen Tierarten viel stärker mit deren Grösse schwanken als die der höheren Pflanzen. Es finden sich in einem Quadratmeter Boden, auf oder in dem von Arten grösserer Insekten wie Käfern, Heuschrecken, Wanzen, aber auch anderen Tieren gleicher Grössenordnung wie Spinnen, Asseln und Regenwürmern, Individuenzahlen in der Grössenordnung von 1-100 vorzukommen pflegen, Collembolen, Milben und besonders Nematoden in solcher Dichte, dass die Individuenzahlen der einzelnen Arten bis 100 000 und sogar bis über 1 000 000 steigen (vgl. hiezu auch die von Frenzel (1936) angegebenen Individuenzahlen). Demgegenüber beanspruchen grössere Tiere wie vor allem die Landvertibraten zumeist einen wesentlich grösseren Minimalraum als 1 m², sodass sie auch wenn einzelne ihrer Arten eine Assoziation verhältnismässig dicht besiedeln, bei Anwendung der vorgeschlagenen Abundanzskala nie in Erscheinung treten würden.

Wollte man so erheblichen Unterschieden in der Individuenhäufigkeit, wie sie bei der Landtierwelt innerhalb der einzelnen Assoziationen tatsächlich vorkommen, mit einer numerischen Abundanzskala Rechnung tragen, so würde diese mit so hohen Zahlen arbeiten müssen, dass ihre Anschaulichkeit darunter sehr leiden würde. Ich schlage daher einer Anregung von H. Sekera folgend vor, an Stelle der numerischen eine logarithmische Abundanzskala zur Anwendung zu bringen. Dieser kann man ohne weiteres wenigstens grössenordnungsmässig die tatsächliche Individuenzahl pro Flächeneinheit zugrunde legen, indem z.B. die logarithmische Abundanzzahl 1, eine zweistellige, die logarithmische

Abundanzzahl 4 aber eine fünfstellige tatsächliche Individuenzahl anzeigt. Folgerichtig bedeutet dann die Abundanzzahl -0.2 , dass nur ein Hundertstel Individuum auf die Flächeneinheit kommt, oder dass die betreffende Tierart erst in einer Fläche auftritt, die hundertmal so gross ist wie die Flächeneinheit. Numerische Abundanzzahlen von 1 bis 10 Individuen lassen sich in der logarithmischen Skala einfach mit den Zahlen 0.1 bis 0.9 zum Ausdruck bringen, für nur in einem Individuum vorhandener Arten, die aber als gesellschaftsset anzusprechen sind, kann weiterhin das Zeichen + in Verwendung bleiben, für zufällige Irrläufer in der betreffenden Assoziation kann unverändert eine kleine hochgestellte Null als Kennzeichen dienen.

ADENINE DERIVATIVES AND THEIR BIOLOGICAL FUNCTIONS

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(Received 2 March 1939)

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I. INTRODUCTION

OUR knowledge of the occurrence and the significance of certain adenine derivatives in biological material is based on the information gained mainly from chemical investigations directed towards the isolation and purification of these compounds from cells and tissues, and from the study of their behaviour in the whole body, in isolated organs and in specially constructed experimental sets of biological cell-free systems. The latter type of experimental procedure can be described under the name of the "biological test". In this particular field under review the application in research of biological tests has proved of inestimable value, not only owing to the advantage of the use in such experiments of minute quantities of the material studied but also because they have frequently helped to reveal unexpected connexions and links between the reaction under investigation and others taking place simultaneously in the biological medium employed.

In this article I intend to deal first with the distribution in nature and the description of the properties of biologically interesting adenine derivatives, and after that I shall endeavour to represent their position in relation to certain biochemical processes. In some cases a brief historical survey will also be given showing the ways and paths which led, sometimes by curious twists, to the final, often unforeseen, elucidation of the various problems.

As regards the chemical configuration of the substances to be discussed below,

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some of them are comparatively simple compounds, whereas others are large molecules of a more complex structure. The first group includes the nucleoside *adenosine* (adenine-*d*-ribose) and the nucleotides, *muscle adenylic acid* (adenine-*d*-ribose-5'-phosphoric acid), *yeast adenylic acid* (adenine-*d*-ribose-3'-phosphoric acid) and *adenyl pyrophosphoric acid* (adenosine-triphosphoric acid). There is an intermediate group of adenosine-polyphosphoric esters, such as the *di-adenosine-pentaphosphoric* and the *di-adenosine-tetraphosphoric* acid. And finally, we have the group of dinucleotides in which besides adenine (6-aminopurine) we have yet another base such as pyridine or alloxazine (Fig. 1); of these *cozymase* (diphospho-pyridine-adenine-dinucleotide), *Warburg's coenzyme* (triphospho-pyridine-adenine-dinucleotide) and *d-amino-acid oxidase coenzyme* (diphospho-alloxazine-adenine-dinucleotide) are representatives.

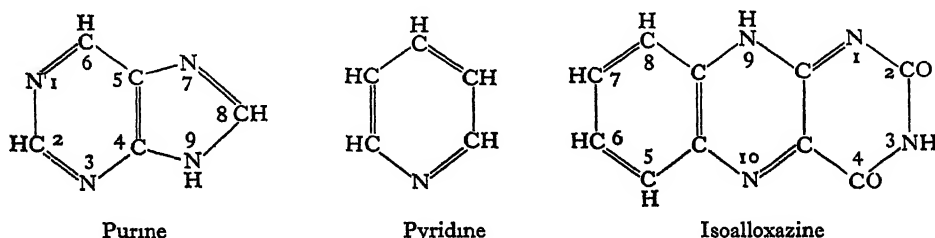


Fig. 1.

II. DISTRIBUTION, ISOLATION AND PROPERTIES OF ADENINE DERIVATIVES

(1) *Adenosine*

Free adenosine does not seem to occur in cells; it can, however, easily be prepared by hydrolysis from yeast nucleic acid as a crystalline, slightly soluble substance, of a molecular weight of 294. This substance has been known to biochemists for a long time but only comparatively recently the discovery was made by Drury & Szent-Györgyi (1929) of the potent pharmacological activity which traces of adenosine produce if allowed to act on the heart and also on some other organs. A comprehensive review on this subject has been written three years ago by Drury (1936), therefore I shall only mention briefly the pharmacodynamical effects of adenosine which also apply to other adenine derivatives although to a much smaller extent. Generally speaking, these substances slow the heart beat or produce complete heart-block, they lower the blood pressure by dilatation of the peripheral vessels, they also have some effect upon the intestine and the uterus. In blood they produce leucopenia followed by leucocytosis.

From the biochemical point of view the explanation of the mechanism of the above-mentioned phenomena proved of great interest, the more so as it became clear from the biological experiments that the physiological activity of adenosine (and similarly of adenylic acid) was in some way related to the amino-group of the purine ring. Deprived of their NH_2 -group by chemical or enzymic hydrolysis and

thus converted into inosine and/or its derivatives, the compounds were no longer capable of producing any of their usual effects. Although it was assumed for some time that an enzymic deamination of adenosine is the cause of the pharmacological action of this substance, Parnas & Ostern (1931) have disproved this hypothesis. At the present moment this problem still lacks a satisfactory explanation in spite of much work on this subject. So far, these two facts are established beyond doubt: (1) to exert the physiological activity the adenine-ribose complex is necessary, adenine itself being totally devoid of action, and (2) the integrity of the amino-group of the purine is in some way essential for the process. I should like to emphasize, however, that in spite of the ignorance concerning the actual mode of action of adenosine on the heart this peculiar and very sensitive biological test for adenine derivatives has been successfully employed as a means of qualitative and quantitative evaluation of very small amounts of adenosine and its derivatives in various tissue extracts and medical preparations (see Drury, 1936, p. 311).

When added to tissue and cell extracts and to body fluids adenosine is found to be deaminated rapidly to inosine in some cases and left intact in others. In addition to liver, a very active adenosine deaminase is present in the heart muscle (Ostern & Mann, 1933) and also in serum (cat's especially, Drury *et al.* 1938), but there is comparatively little of it in the skeletal muscle (Ostern & Mann, 1933). Whereas most of the bacteria studied are a potent source of adenosine deaminase (Lutwak-Mann, 1936), in yeast adenosine does not undergo deamination (Ostern *et al.* 1938). In connexion with the study of the bacterial deamination of adenine it was found (Stephenson & Trim, 1938) that it can undergo deamination only as a riboside, small (catalytic) amounts of adenosine acting as "coenzyme" for this process.

(2) *Adenylic acid*

Almost a century ago Liebig (1847) discovered in meat extract inosinic acid which a few years later was analyzed and found to be a hypoxanthine nucleotide (Haiser, 1895; Levene & Jacobs, 1911). However, its role in the muscle tissue remained obscure for a long time. Meanwhile, Bass (1914) reported the presence in human red cells of an organic acid-hydrolysable adenine complex, and very soon afterwards Embden & Laquer (1914-15), who were at that time engaged in the purification and identification of lactic acidogen from muscle press-juice, accidentally made the observation that one of the fractions of their muscle preparation consisted of an organic phosphoric acid compound of adenine and ribose. The isolation of an adenine nucleotide from plant nucleic acid (Jones & Kennedy, 1918) and several improvements in the technique of purification and estimation of the nucleotides have enabled investigators to study more closely the distribution of the adenine nucleotide in biological material. It was found to be a common cell constituent (Jackson, 1923; Jones & Perkins, 1924; Buell & Perkins, 1928), and soon afterwards a number of important observations were made from which gradually emerged the conception of the general significance and the role of adenylic compounds in the enzymic organization of the cell.

Parnas & Heller (1924), when studying and discussing the possible sources of ammonia formation in blood, were the first to suggest the adenine nucleotide as the probable mother substance of blood ammonia. Soon afterwards Embden & Zimmermann (1927) succeeded in isolating adenylic acid (adenine nucleotide) from muscle and associated its presence in muscle with the old finding of Liebig, by assuming inosinic acid to be the product of enzymic deamination of adenylic acid. Simultaneously, experiments were described by Parnas & Mozołowski (1927) in which these authors found that when muscular tissue is ground with water or saline a large ammonia formation occurs ("traumatic ammoniogenesis") which can be prevented by the use of borate instead of the other media. The authors regarded the formation of ammonia as due to the deamination of a substrate present in the muscle by enzymes liberated from the cells by grinding, recalling a somewhat similar traumatic effect in muscle accompanied by lactic acid formation shown much earlier by Fletcher & Hopkins (1907). Next, by the work of Embden & Parnas and their respective co-workers on the ammonia and lactic acid production at various stages of muscle contraction, the fact became firmly established that deamination of adenylic acid is responsible for the ammonia formation observed during exhaustive muscle contractions. Resynthesis of adenylic acid was demonstrated in periods of recovery, not, as was at first assumed from the ammonia given off in the course of the activity (Embden), but from some other, hitherto unknown source, most likely from aminoacids (Parnas). In those early stages of research in this field attempts were already made to discover the relationship between the breakdown of carbohydrate as measured by the production of lactic acid and the formation of ammonia in muscle (Embden & Wassermeyer, 1928; Chrzyszczewski & Mozołowski, 1928); however, the results obtained at that time allowed of no conclusive statements.

The remarkable discoveries of the part played by the adenylic acid in "traumatic ammoniogenesis" in various forms of rigor and in contraction of the muscle aroused the interest of biochemists; in fact, one may say, they started a perfect avalanche of experimental research work in the biochemistry of the adenine derivatives, at first in muscle only, but rapidly spreading to other fields previously only very vaguely or not at all connected with each other, such as the yeast (and bacterial) fermentation and biological oxido-reductions.

Research on adenylic acid was greatly facilitated by the excellent method of Ostern (1932) for large-scale preparation of the pure nucleotide from horse meat which has been adopted for commercial purposes. At the same time the adenine nucleotide obtained from yeast nucleic acid was available, and comparative studies on these two adenine nucleotides led to very important findings. Although composed of the same units (adenine, ribose, phosphoric acid, molecular weight 360), the muscle and the yeast adenylic acids were shown to differ essentially, both in chemical and in biological properties. Muscle adenylic acid is more soluble in water, has a higher melting-point (200° as against 195°) and a smaller laevorotation than the yeast adenine nucleotide; on hydrolysis it yields only about 10% of the theoretical value of furfural and the yeast compound as much as 75%. The phosphoric acid

group is much more resistant to acid hydrolysis in muscle adenylic acid than in the other. Unlike the yeast nucleotide, muscle adenylic acid does not yield formaldehyde on treatment with periodate in acid solution. The relationship of the above differences to the internal configuration of the respective molecules was given by Klimek & Parnas (1932). These authors found that muscle, but not yeast, adenylic acid, forms a copper complex soluble in alkali and insoluble in excess calcium hydroxide, a property typical of polyvalent alcohols. This and other tests (by Levene *et al.* 1931, 1932, 1933) furnished strong evidence that two free *neighbouring* hydroxyl groups exist in the muscle adenylic ribose, the phosphoric acid being attached to C_5 of ribose, whereas in the yeast adenine nucleotide it is situated on C_3 of the ribose molecule¹ (Fig. 2).

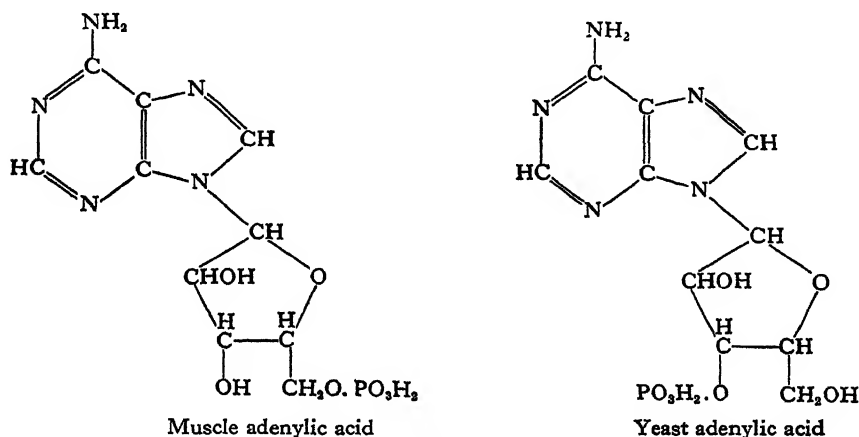


Fig. 2.

It was felt by some workers that these two nucleotides, obviously so different, should have names which would obviate the use of their long chemical formulae and still distinguish between them, especially after the discovery was made of the existence of yeast adenylic acid in an animal tissue (pancreas) and of muscle adenylic acid in yeast. Therefore some authors proposed to affix t- for the animal, and h- for the yeast product, others used the prefix erg- for the muscle and syn- for the yeast adenylic acid (Lindner, 1933). However, these suggestions have not been accepted generally and have found little application in the literature. I propose to use MA and YA in this article as convenient abbreviations for the muscle and yeast adenylic acid, respectively.

Valuable confirmation of the distinction in the structure of the two adenylic acids is also derived from the study of their behaviour under the action of enzymes from various sources. It was invariably found that the rates of decomposition (by dephosphorylation and deamination) of the two substances by a given tissue or cell extract differed greatly from each other, thus pointing to the high specificity of the enzymes concerned. (Schmidt, 1928; Ostern & Mann, 1933; Lutwak-Mann, 1936).

¹ Both the muscle and the yeast adenylic acids are *ribose* derivatives, unlike the adenine nucleotide from the animal nucleic acid which is a *desoxyribose* derivative.

During the investigations of the kinetics of the deamination of MA by muscle extracts an observation was made which gave the first clear indication of a *material link* between the ammonia formation and the glycolysis in muscle. It was noticed (Mann, 1933) that in a few-days-old and glycolytically inactive extract deamination proceeded much more rapidly than in fresh extracts in which the glycolytic power was still preserved. As will be seen in later sections (see p. 413), ample use was made in the future of this observation in a series of experiments designed to establish the role of the adenylic system in the glycolysis cycle.

(3) *Adenyl pyrophosphoric acid (adenosinetriphosphoric acid, ATP)*

When studying the rate of acid hydrolysis (N HCl, 100° C.) of the phosphorylated carbohydrates in muscle, Lohmann (1928*a*) found that the first 10 min. of hydrolysis are accompanied by a rapid formation of inorganic phosphate due to the dephosphorylation of an easily hydrolysable phosphoric acid derivative. On closer examination of the properties of this acid-sensitive fraction of the muscle it was identified and isolated as pyrophosphate (Lohmann, 1928*b*). By means of P-estimations this fraction was shown to exist in practically every cell and tissue both of animal and plant origin, striated muscle and yeast being among the richest. For some time no explanation was available with regard to the significance of the pyrophosphate in muscle. It was known, however, that this unstable phosphoric compound was responsible to a certain degree for the accumulation of inorganic phosphate on muscle autolysis or after strenuous muscular contractions. No connexions with MA were suspected at first. Inorganic pyrophosphate, however, when added to muscle preparations repeatedly failed to show behaviour identical with the preformed muscle pyrophosphate and so, lacking a better explanation, some special undefined configuration of the preformed compound was postulated (Lohmann, 1928*b*).

Later, after a more thorough study, Lohmann (1929) found that in the muscle (and other tissues as well) the pyrophosphoric group is combined with adenylic acid. The instability of this complex in alkali explained why Embden (1927), who used calcium hydroxide in his method, obtained from muscle the MA only. Almost simultaneously with Lohmann's discovery the existence of an adenosine triphosphoric complex in muscle was announced by Fiske & Subbarow (1929). The general occurrence of ATP in cells and tissues other than rabbit and frog muscle was established by means of both analytic and preparative methods (red cells: Barrenschén & Filz, 1932; Warburg & Christian, 1936*b*; Lohmann & Schuster, 1937; bacteria: Lutwak-Mann, 1936; Mesrobianu, 1936; yeast: Lutwak-Mann & Mann, 1935; Wagner-Jauregg, 1936; invertebrates: Lohmann & Schuster, 1934). But in practice rabbit skeletal muscles have mostly been used for the isolation of the ATP on a large scale, preferably in the form of the amorphous Ba-salt (Lohmann, 1931*b*) or Ag-salt (Barrenschén & Filz, 1932). Crystalline acridinium compounds of ATP were described by Wagner-Jauregg (1936*b*).

Neutral or alkaline hydrolysis of ATP causes its decomposition into MA and

pyrophosphate. Acid hydrolysis yields adenine, ribosephosphate and orthophosphate. The molecular weight of ATP was found to be 413-485 from freezing-point determination, which may be due to partial decomposition as it is theoretically 506. The chemical structure of ATP was the object of much discussion and controversy especially as regards the position of the pyrophosphate in the molecule; for some time also uncertainty existed about the link between the purine and the ribose rings. The formula generally accepted to-day as a result of both chemical and enzymic studies, is one where the three phosphoric acids are esterified with each other at the C_5 of ribose (Lohmann, 1935*b*). The suggestion of Barrenschen *et al.* (1933) of the pyrophosphate being attached to the adenine amino-group was disproved by the fact that inosine pyrophosphate was obtained from ATP by deamination with nitrous acid (Lohmann, 1932; Mozołowski & Sobczuk, 1933) as well as by studies on the enzymic decomposition of ATP. The link between purine and ribose is in position N_9 of adenine and not as was thought before, in N_7 (Gulland & Holiday, 1936).

Much work has been done on the enzymic decomposition of the ATP, in muscle and in other tissues and cells. Enzymes which vigorously attack this substance were found to be present in most of them, the mechanism of their action (for some time a matter of controversy between Lohmann and Mozołowski) being generally the hydrolysis of the pyrophosphate to orthophosphate with the subsequent deamination of the MA. In dialysed extracts of lobster muscle Lohmann (1935*a*) found a two-step enzymic hydrolysis of the pyrophosphate. One phosphoric acid is split off first, thus leaving adenosine-diphosphate (ADP) which can be isolated as a Ba-salt of a solubility somewhat different both from the MA- and ATP-Ba-salts. In the second stage, ADP undergoes dephosphorylation to MA but only in the presence of Mg, a fact which suggests two different enzymes being engaged in the breakdown of ATP.

In order to pursue the fate of ATP in the muscle during autolysis and at various stages of muscle activity a method for quantitative determination was necessary. There was, of course, Lohmann's rapid and simple method of P-estimation after acid hydrolysis. However, although accurate if applied to pure solutions of the ATP, it required many corrections when ATP was estimated in the muscle in presence of several other more and less easily hydrolysable phosphoric acid derivatives. Furfural estimations were of little use, since this involved a tedious procedure giving too low values. A method was therefore devised by Parnas & Lutwak-Mann (1935) for the estimation in muscle of ATP, MA and ammonia, the sum of which represents the state of the adenylic system at any given moment. In this method use was made both of the chemical properties of the substances concerned as well as of their decomposition by the muscle enzymes. ATP and MA are first separated completely by means of barium and next the content of the two fractions of adenine-amino-nitrogen (NH_2-N) is assessed by the use of small quantities of frog muscle suspension (100 mg. muscle) as the source of enzymes which dephosphorylate ATP and deaminate MA. Free ammonia formed in the course of the experiment is estimated directly by distillation (Parnas & Heller, 1924). By means of

this method it could be shown that in fresh skeletal muscle MA does not occur in free form but that all of it is esterified to ATP (Parnas & Lutwak-Mann, 1935; see also Lohmann & Schuster, 1934), a fact which probably applies to most cells and tissues (neither does free pyrophosphate occur in tissues, Umschweif & Gibaylo, 1937). Although the method was originally planned for muscle it was later successfully applied to other biological material (yeast: Lutwak-Mann & Mann, 1935; bacteria: Lutwak-Mann, 1936).

Heart muscle seems to represent a peculiar type of tissue with regard to the state of its adenine nucleotide. It was mentioned above that heart enzymes deaminate selectively adenosine (see p. 401), which may explain the presence of free MA in this tissue. Moreover, it was first observed by Embden (1932) and later confirmed by Beattie *et al.* (1934) and Ostern (1934), that the nucleotide from heart muscle has the composition of a diadenosine-pentaphosphoric acid, an extremely labile complex which, except under special precautions, easily breaks down to a mixture of ATP and ADP. So easily does this occur that by Lohmann & Schuster (1934) its existence has been denied altogether. In view, however, of the isolation of similar adenosine-polyphosphoric compounds from yeast (Wagner-Jauregg, 1936), from red cells (Warburg & Christian, 1936*b*), and more recently of di-adenosine-tetraphosphate (ATP+MA) from yeast (Kiessling & Meyerhof, 1938), evidence seems to accumulate in favour of the occurrence of various complex combinations of the adenine nucleotide.

(4) *Cozymase (coenzyme of alcoholic fermentation, coenzyme I, diphosphopyridine nucleotide)*

Three facts stand out in the long story of the development of our chemical knowledge of cozymase: (1) the discovery of Harden & Young (1904) of the existence of a thermostable and dialysable factor in yeast juice the addition of which produced a marked increase in the rate of sugar fermentation in yeast preparations, previously freed from the coenzyme by ultrafiltration; (2) the establishment by Euler and his collaborators (1928-31) of the adenine nucleotide configuration of cozymase; and (3) the final demonstration that the chemical structure of cozymase is a dinucleotide of two bases, pyridine and adenine (Warburg *et al.* 1935*a*; Euler *et al.* 1936), a sequel to the earlier discovery of the pyridine ring (nicotine acid amide) in Warburg's coenzyme from red cells (Warburg & Christian 1935; see below, p. 409).

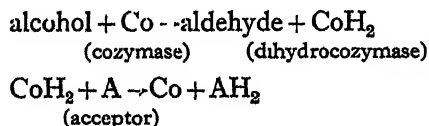
In spite of the long period which separated the discovery of cozymase as the coenzyme of alcoholic fermentation from the recognition of its true structure, much valuable information has been gained in the meantime about the general distribution of cozymase, its methods of isolation and some of its properties. Attempts were made to elucidate the mode of action of cozymase in enzymic reactions.

As the source of cozymase for large-scale preparations, baker's yeast is generally used (Myrbäck & Euler, 1931; Euler *et al.* 1936; 5 g. pure substance from 100 kg.), or bottom yeast (Meyerhof & Ohlmeyer, 1937); but muscle (Ochoa, 1937), red cells (Warburg & Christian, 1936), liver (Dixon & Lutwak-Mann, 1937; Lutwak-Mann,

1938), also bacteria (Myrbäck & Euler, 1925; Yudkin, 1933) and plants (Euler & Steffenburg, 1928) contain fair quantities. At the time when the chemical configuration of cozymase was still unknown special tests were used for the demonstration of its presence in various materials and also for the determination of the degree of purity of cozymase preparations. Although to-day mostly of historical value, the usefulness of those tests in earlier work seems to justify a brief description. Thus for instance, Euler has expressed the activity of his cozymase preparations in so-called "A-Co units" (apozymase-cozymase) of which one unit represented the amount of cozymase which under certain standard conditions of yeast sugar fermentation caused a 1 cm.³ CO₂ output per hr. per g. of dry weight. Euler's most active preparations had 600,000 A-Co units. The principle of the method was simple, but considerable difficulties were experienced in the preparation of active coenzyme-free "apozymase" from exhaustively washed, dried yeast.

An extremely sensitive system for the demonstration of traces of cozymase is *B. parainfluenzae* (Lwoff, 1937). Several oxido-reductive enzyme systems such as the lactic dehydrogenase, aldehyde mutase and many others, can also be prepared cozymase-free and then used for an approximate evaluation of cozymase. By means of the various biological tests the wide distribution in nature and the identity of cozymase independently of its origin was shown. On the other hand, it was fully realized that cozymase could not be replaced by ATP in enzymic processes.

Already before the actual structure of cozymase became established convincing evidence had accumulated to the effect that cozymase is a specific coenzyme of enzymic oxido-reductions. The key to the mechanism was offered by the discovery of the pyridine ring in Warburg's coenzyme and by the elucidation of its action as "hydrogen transferring" agent, in this coenzyme first, and next in cozymase itself (Warburg & Christian, 1936a; Euler & Adler, 1936). In the enzymic oxido-reduction system the role of the pyridine part of the cozymase molecule consists in the transfer of hydrogen from the activated substrate to a hydrogen acceptor,¹ thereby undergoing alternate reduction and oxidation (pyridine \rightleftharpoons dihydropyridine, cozymase \rightleftharpoons dihydrocozymase). The oxidation of alcohol to acetaldehyde by the enzyme alcohol dehydrogenase (yeast, liver, sugar beet, bacteria) may serve as a simple example to demonstrate the reversible oxido-reduction of cozymase and at the same time the character of cozymase as a true catalyst, with a flavoprotein (see below, p. 410) to be placed in the position of the hydrogen acceptor.



Oxidized cozymase (Fig. 3) is relatively stable in acid solution but is rapidly inactivated by alkali, whereas the dihydrocozymase behaves in the reverse way, which is due to the sensitivity to acid of the partially hydrogenated pyridine ring. Cozymase

¹ For a general discussion on biological oxido-reduction systems see Dixon, 1929 (*Biol. Rev.*).

prepared from yeast or any other source is practically wholly in the oxidized form although, of course, in the cells both the reduced and the oxidized forms exist in varying ratios. Reduced cozymase can be obtained from the oxidized form not only by enzyme action but also simply by reduction with hyposulphite in mildly alkaline medium. A method has been worked out (Ohlmeyer, 1938) for the preparation of bigger quantities of dihydrocozymase as a sodium salt. Dihydrocozymase is perfectly stable, non-autoxidizable and requires for oxidation a special enzymic hydrogen carrier (flavoprotein). Cozymase has an absorption spectrum in the ultra-violet due to adenine and pyridine, with a maximum for oxidized cozymase at $260\text{ m}\mu$., whereas the reduced form prepared enzymically or by hyposulphite has yet another very strong absorption band at $345\text{ m}\mu$. called the "dihydro-band". Very small quantities of cozymase are sufficient for optical examination, hence the wide application of the study of absorption spectra in the course of enzyme reactions involving cozymase. Solutions of dihydrocozymase show white fluorescence in ultraviolet light. The phenomenon disappears (reversibly) on oxidation.

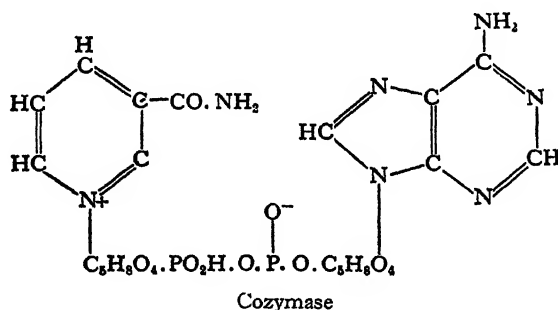


Fig. 3.

Hydrogenation of cozymase by means of platinum and hydrogen causes an uptake by the pyridine ring of 3 mol. of hydrogen instead of one as in the enzymic or hyposulphite hydrogenation. The substance thus obtained, hexahydrocozymase, has no catalytic activity in biological oxido-reductions and it no longer shows the typical absorption band at $345\text{ m}\mu$., since the pyridine has been converted to piperidine which has no spectrum either in this or in the $260\text{ m}\mu$. (adenine) region.

Deamination of cozymase by the muscle deaminase or by nitrous acid causes an appreciable fall of its activity (Euler *et al.* 1937).

Free nicotinic acid amide cannot be partially hydrogenated either chemically or enzymically and cannot replace cozymase. It is, however, a substance of great biological interest: it has vitamin activity in pellagra and rat "black tongue" (Frost & Elvehjem, 1937) and also acts as a growth factor in certain bacteria (Knight, 1937). With regard to the effect of nicotinic acid deficiency on the cozymase content of tissues see Axelrod & Elvehjem (1939). Of other pyridine derivatives the plant alkaloid trigonellin (methyl betain of nicotinic acid) undergoes partial hydrogenation by hyposulphite in alkaline solution and shows the typical dihydro-band at $350\text{ m}\mu$. However, it is inactive in enzyme systems.

(5) *Warburg's coenzyme ("hydrogen transferring" coferment, coenzyme II, triphosphopyridine nucleotide, phosphocozymase)*

In connexion with studies on glucose oxidation in haemolysed red cells Warburg & Christian (1931) discovered an enzyme which they called the "Zwischenferment", capable of the oxidation of hexose-6-monophosphate (Robison ester) to phosphohexonic acid in the presence of a coenzyme. An identical system was found also to be present in Lebedew's yeast maceration juice from which both enzyme and coenzyme were isolated in purified form (Warburg & Christian, 1932). In the course of chemical analysis of the new coenzyme the adenylic structure of it became established, and also the presence of a second base apart from adenine was made probable (Warburg & Christian, 1934, 1935). Shortly afterwards, the base was identified as the amide of nicotinic acid (Warburg *et al.*, 1935*a, b*) and the coenzyme stated to contain: 1 adenine, 1 pyridine (nicotinic acid amide), 2 ribose and 3 phosphoric acid molecules (mol. wt. — 743). In the phosphoric acid content lies the sole difference from cozymase, which has two phosphoric acids only (mol. wt. 681). However, in spite of the close similarity the two coenzymes are strictly specific; cozymase has no catalytic activity in the hexosemonophosphate dehydrogenase system and the addition of triphosphopyridine nucleotide does not increase the rate of the alcoholic sugar fermentation or muscle glycolysis. However, when acted upon by yeast enzymes for a longer time, the triphosphopyridine nucleotide is converted into cozymase (to a small extent only) and subsequently it gives the usual cozymase effect (Euler & Adler, 1938). For other systems apart from the hexosemonophosphoric ester dehydrogenase in which this dinucleotide acts as coenzyme, see below (p. 415).

The mechanism of Warburg's coenzyme with regard to hydrogen transfer is due to the pyridine ring of the substance and is precisely the same as in cozymase (p. 407), except, of course, that it acts in presence of a different enzyme and substrate. The same is true of other properties such as absorption spectrum and pH sensitivity, and therefore need not be repeated. But it should be remembered that all these essential facts concerning the nature of pyridine nucleotides were first established by Warburg & Christian for their coenzyme, and subsequently by simple analogy, for cozymase.

By means of Warburg's hexosemonophosphate dehydrogenase test the occurrence of the triphosphopyridine nucleotide was demonstrated in several cells and tissues. As the most suitable source for large-scale preparation and purification of the coenzyme, blood (red cells) is used preferentially (Warburg & Christian, 1936*b*), from 1000 l. of which 1.0–2.0 g. of pure substance can be obtained, and not yeast, because of the difficulty in this case of separation from cozymase.

(6) *Coenzyme of the amino-acid oxidase (alloxazine-adenine dinucleotide)*

The *D*-amino-acid oxidase is an enzyme present both in animal tissues and in yeast (Krebs, 1933, Das, 1936). In the course of the purification of this system the probability of a coenzyme was suggested by Das (1938). Warburg & Christian

(1937-8) succeeded in separating the enzyme from the coenzyme which they reported shortly afterwards (1938*a*) to contain flavin and adenine. An identical observation about the flavin nature of the coenzyme, and details of the purification from heart were published shortly before by Straub (1938). The analytically pure coenzyme was finally obtained from kidney by Warburg & Christian (1938 *a, b*) as an orange brown Ba-salt and shown to consist of riboflavin-phosphoric acid linked with adenylic acid ($-1 \text{ H}_2\text{O}$), mol. wt. 785. Later, the flavin-adenine dinucleotide was also isolated from yeast (Warburg *et al.* 1938) and found to be identical with the animal product. Heart muscle and liver contain about 50 mg., kidney, baker's yeast about 20 mg. and skeletal muscle 3 mg., of this dinucleotide per kg. wet weight. The alloxazine-adenine nucleotide is completely stable in aqueous neutral solution, but fairly sensitive to acid and alkali. On treatment with alcohol or acetone the substance undergoes destruction accompanied by the appearance of a greenish fluorescence. Optically the alloxazine-adenine nucleotide gives spectra very similar to lactoflavin both in the visible and in the ultra-violet region.

In the amino-acid oxidase system the mechanism of the coenzyme consists of reduction of the alloxazine ring by the substrate, followed by the oxidation of the dihydroalloxazine by molecular oxygen. However, the flavin-adenine dinucleotide exists also in tissues completely devoid of amino-acid oxidase activity, e.g. in heart muscle. This fact suggests that, like the pyridine-adenine nucleotides, it acts as a carrier of other, perhaps of several, enzymic systems as well. This is especially probable in view of the existence in yeast of the "yellow oxygen transferring enzyme" (flavoprotein), the active group of which has been shown by Warburg & Christian to be an alloxazine *mononucleotide*. Other flavoproteins have already been isolated and found to contain the flavin-adenine dinucleotide, from milk by Corran & Green (1938), yeast by Haas (1938) and heart muscle by Straub (1939); this last is assumed by Straub *et al.* (1939) to be identical with the "coenzyme factor" of Dewan & Green (1937) and the "diaphorase" of Adler *et al.* (1937). Owing to the high specificity of the enzymic protein part of those flavoproteins they can be separated chemically from each other in the course of the preparation; their properties differ also with regard to their ability to react with oxygen. Generally speaking, their part in the oxidation-reduction systems is to carry the hydrogen from the dihydropyridine nucleotides to oxygen or to some other hydrogen acceptor (methylene blue). There are indications that the xanthine oxidase of milk also requires the flavin dinucleotide for its action (Ball, 1938).

III. ADENINE DERIVATIVES IN BIOLOGICAL PROCESSES

To give an illustration of the biological role of adenine derivatives I shall give some examples of the part which they play in the cycles of muscle glycolysis and yeast fermentation and also in other enzymic systems. In distinction from other branches of biochemical research where tissue slices or intact cells (blood, yeast, bacteria) serve as experimental material, most of the knowledge of the above-men-

tioned processes is based on experimental evidence gained from the study of *cell-free* enzymic preparations.

No discussion can be entered on here concerning the suitability for biological purposes of such apparently "artificial" systems. In several of the general reviews on glycolysis and fermentation (see References) this problem has been exhaustively dealt with and excellent reasons put forward to explain and to support the practice of using preparations for experiments in which the cell structure has been destroyed by mechanical or chemical means, and also of operations such as autolysis, dialysis, the use of specific enzyme poisons (e.g. iodoacetate, fluoride, borate, phloridzine), "radioactive" phosphorus, etc.

Somewhat as in a deliberately slowed down or accelerated motion picture, sections of complicated processes can be conveniently analysed in those "artificial" experimental systems, which otherwise would escape our perception owing to the lack, at present, of more suitable means of investigation *in vivo*.

In the cycles of glycolysis and fermentation the adenine derivatives play a prominent part in closely interlocked reactions of the following type: adenosine, and the mononucleotides, adenylic and adenylic pyrophosphoric acid, are involved in phosphorylations (phosphate transfer); cozymase, the dinucleotide of pyridine and adenine, in the oxido-reductions (hydrogen transfer). Apparently, the mono- and the dinucleotide are not interchangeable: pure cozymase does not act as a phosphate acceptor or donator. However, alkali-inactivated cozymase, hexahydro-cozymase, and also cozymase itself in presence of Mn may to a certain degree replace the mononucleotides ("cophosphorylase") in their specific function. Likewise, a mixture of adenylic pyrophosphate with nicotinic acid amide has no "hydrogen carrier" activity. Neither can the triphosphopyridine dinucleotide be substituted for cozymase, in spite of their close chemical relation, which is perhaps partly due to the fact that in cell-free enzyme systems these two have been found to be interconvertible only to a very small extent (Euler & Adler, 1938). Although biologically of great interest, the problem of the interconvertibility of the dinucleotides requires a great deal more study than is at present available, especially as regards the behaviour of those substances when acted upon by tissue enzymes.

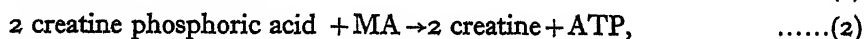
By a few deliberately chosen, simplified examples given in approximately historical order, I hope to convey to the reader how, starting from various stand-points, the conception was gradually built up step by step, of the adenylic compounds acting as the phosphate transferring system in glycolysis and fermentation, and of the close relationship between phosphorylations and oxido-reductions in those two processes; also of oxido-reduction systems other than the glycolysis and fermentation cycles which depend upon adenine derivatives.

(1) *Phosphate transfer*

(a) *ATP in the role of "coenzyme"*. A fresh muscle *Brei* prepared by grinding muscle with a small quantity of water or dilute phosphate rapidly produces lactic acid from added glycogen. However, after a few hours' autolysis it loses its glycolytic power. Lohmann, continuing the work of Meyerhof in this field, found that

the glycolysis can be restored to the former level in such inactivated preparations by the addition of small quantities of boiled muscle juice or yeast juice (*Kochsaft*). If, for the preparation of the reactivating boiled juice, old autolysed muscle is used instead of a freshly ground muscle, no effect on glycolysis can be achieved. The autolysable "co-factor" of glycolysis has been identified by Lohmann (1931*a*) as ATP. Instead of muscle *Brei*, homogeneous muscle extracts can be used which show a behaviour analogous to the *Brei*, but if dialysed they will not glycolyse glycogen even on addition of ATP. Magnesium and inorganic phosphate were found to be required in addition to ATP in order to restore the activity of autolysed and dialysed extracts (Lohmann, 1931*b*). Thus Lohmann's "coenzyme of lactic acid production" was composed of an autolysable organic factor, the ATP, and two non-autolysable inorganic compounds, the magnesium and phosphate ions. Similar findings were made soon afterwards in the glycolytic system of red cells (Meyerhof, 1932). ATP in these experiments, could not be replaced by cozymase and vice versa, it could not be substituted in alcoholic fermentation for cozymase, although it was known that addition apart from cozymase, of ATP, magnesium and phosphate favourably increased the rate of carbon dioxide production. Thus the necessity was established for the presence of the adenylic system for lactic acid production from glycogen (and hexosephosphates, Lohmann, 1931*b*), but not the mechanism of this "coenzyme" activity. The closer study in the years that followed this discovery of the various intermediary reactions known to occur in the course of autolysis and also in muscle contraction helped to reveal more of the true role of adenylic compounds in glycolysis.

Creatinephosphate (phosphagen) was discovered in vertebrate muscle by Fiske & Subbarow (1927) and by Eggleton & Eggleton (1927); invertebrate muscle was found by Meyerhof & Lohmann (1928) to contain arginine phosphate instead, both substances very similar to each other in almost every respect. Early experiments have shown that traumatic destruction of the muscle structure or exhausting contractions cause the phosphagen to break down rapidly and yield inorganic phosphate (hence the name). At the same time some connexion was anticipated between the integrity of the creatinephosphate in muscle and the adenylic system (see e.g. Lundsgaard, 1930; Mozolowski *et al.* 1931). A new turn in this problem occurred when Lohmann found (1934) that while the enzymic breakdown of creatinephosphate in long autolysed aqueous muscle extracts was exceedingly slow (which was at first taken to be due to enzyme inactivation), it could be greatly accelerated by the addition of boiled fresh muscle juice. ATP was identified as the component of the juice responsible for the activation of the decomposition of phosphagen. Later Lehmann (1935) found that magnesium is also essential for this reaction. Lohmann pictured the course of the phosphagen breakdown in the following way ("Lohmann reaction"):



which shows how catalytic amounts of MA can bring about dephosphorylation of

large quantities of creatinephosphate. In Lohmann's experiments the ATP was added to the extract to yield the necessary MA, however it was shown later that actually, in the course of glycolysis, ATP is continuously formed by synthesis from MA and one of the phosphorylated carbohydrate esters (see p. 414).

Experiments similar in kind to those described above were carried out by Lohmann & Meyerhof (1934) on the dephosphorylation in cell-free extracts of phosphoglyceric (phosphopyruvic) acid which is an important intermediary of the carbohydrate breakdown both in glycolysis and in fermentation. The decomposition of phosphoglyceric via phosphopyruvic acid (for the enzymic components of this reaction, see Baranowski, 1938) proved to be not a simple hydrolysis into phosphoric and pyruvic acid but to require MA (and Mg) as "coenzyme" which at that time was still the common denomination for the role of the adenylic system. Below I shall try to describe how, in the course of a special type of experimental procedure, this rather theoretical conception had in the end become transformed into the perfectly concrete formulation of the MA/ATP system as an alternate phosphate acceptor and donator in the intermediate phases of glycolysis and fermentation.

(b) *Integrity of ATP as function of glycolysis.* For the proper interpretation of the experimental examples quoted below a brief recapitulation of some characteristics of the muscle "ammonionogenesis" largely developed by Parnas and his collaborators (1927-37) will be of help to those not quite familiar with this problem. In muscle ground with a large quantity of water (10 vol.), in the course of about 30 min., 6-10 mg. of ammonia-nitrogen are formed from the adenylic acid content of the muscle (frog). Muscle ground with only one volume of water or with phosphate shows no ammonia production for a certain time (*ca.* 30 min.), after which period the values of the diluted *Brei* will be gradually reached (Parnas, Ostern & Mann, 1934a). This "preventive" effect of phosphate on the deamination of MA can be completely abolished by iodoacetate or fluoride, both of which are known as specific poisons of certain stages of the glycolytic and fermentative breakdown of carbohydrates. Taken together, (1) the transient effect of phosphate on the adenylic system (i.e. by no means a poisoning of the specific deaminase), (2) the effect of typical glycolysis poisons on the integrity of the adenylic system, (3) the previously mentioned observation of Mann (1933, see above p. 404) that glycolytically inactive extracts deaminate ATP much more vigorously than fresh ones in which glycolysis is in progress, and (4) the fact that it is in the form of MA in which ATP undergoes deamination in muscle, led Parnas and his co-workers to the following conclusions. The state of the adenylic system in muscle as expressed by the ammonia formation, is closely linked with the glycolytic breakdown of carbohydrate. The existence of a *material* and not merely energetic link, was proved by experiments in which the effect of various intermediary components of the glycolytic cycle was tested on the ammonia formation in muscle. If the addition of a certain phosphorylated glycolysis product causes a *decrease* in ammonia formation, it means that this substance acts as a phosphate donator for MA, thus preventing deamination, whereas MA, the acceptor, at the same time catalyses the dephosphorylation of this substance. On the other hand, *increase* of ammonia production is an

indication of a substance acting as phosphate acceptor for the labile groups of the ATP, which in this case is in the role of a donator of phosphate and, *eo ipso*, acts as a phosphorylating catalyst.

Out of a long series of experiments I shall only briefly describe a few to illustrate the above statements. It was found by Parnas *et al.* (1934*b*) that addition of phosphopyruvic acid prevents the deamination of MA, simply because of the phosphorylation of MA by phosphopyruvic acid to ATP which is not deaminated in muscle ("Parnas reaction"). The same reaction, only from a different point of view, has already been discussed above (p. 413). It will be remembered that Lohmann (1934) has shown ATP to be linked with the creatine-phosphate breakdown. Now, by mixing in a rabbit skeletal muscle extract (in presence of magnesium), phosphoglyceric acid, MA and creatine, a synthesis results of creatine phosphate, owing to the action of MA as a phosphate acceptor for the glycolytic product on one side, and as a donator of phosphate for the phosphagen on the other (Ostern *et al.* 1935; Needham & v. Heyningen, 1935; Lehmann, 1935). In an identical manner, the arginine phosphagen can be synthesized in an extract of invertebrate muscle (Lehmann, 1936.)

The reaction between hexosemonophosphoric acid (Embden ester) and ATP provides an instance of an opposite character. The addition of this ester to an inactivated muscle extract causes an increase in ammonia formation, without, however, an accumulation of free inorganic phosphate, because the phosphate from the ATP (donator) is directly transferred to the monoester (acceptor) to form the hexosediphosphoric ester of Harden & Young (Ostern *et al.* 1936).

Phosphate transfer on lines similar to or approaching those sketched above for skeletal muscle, occurs in many animal tissues (heart muscle, Ostern *et al.* 1935; red cells, Dische, 1936*a*; kidney, Kalckar, 1938; brain, Meyerhof, 1939; electric organ of *Torpedo*, Baldwin & Needham, 1936), at least in certain types of tumour (Boyland *et al.* 1937; Holmes, 1937). However, in the embryo (Needham & Lehmann, 1937), and possibly in brain (Ashford & Holmes, 1929; Ashford, 1934), also a non-phosphorylating mechanism of carbohydrate breakdown exists.

(c) *Phosphorylation in yeast fermentation.* For studies of the cell-free alcoholic fermentation either plasmolysed dried yeast or Lebedew's maceration juice from dried yeast are most frequently used. The adenine derivatives in these preparations undergo only dephosphorylation and not deamination. Transfer of phosphate via the adenylic system, though different in some particulars from the muscle scheme, has been shown to occur in Lebedew's juice by Lutwak-Mann & Mann, 1935; Ohlmeyer, 1935; Euler & Adler, 1935; Schäffner & Berl, 1936. Not only MA but also adenosine can act in yeast phosphorylations as an acceptor of phosphate (Ostern *et al.* 1937, 1938).

Phosphorylation by *living* yeast of MA (Neuberg, 1935; Lutwak-Mann & Mann, 1935) and of adenosine (Ostern *et al.* 1937, 1938), to adenosinepolyposphoric esters, deserves to be mentioned here because doubts have been expressed by some authors (Nord *et al.* 1936) as to whether fermentation by living yeast cells involves a phosphorylating mechanism at all.

For information concerning similar processes in bacteria see Stephenson (1939) on fermentation of hexoses.

(2) *Hydrogen transfer*

The mechanism of cozymase activity as the coenzyme of certain enzymic oxido-reductions has been explained in a previous section (p. 407) as due to the pyridine group. Stress has been laid upon the specificity and non-interchangeability of this coenzyme. Below, reactions will be discussed which involve hydrogen transfer by cozymase, some of which represent essential stages of glycolysis and fermentation and others which are not connected with these two processes.

(a) *Oxido-reductions coupled with phosphorylation.* In the course of carbohydrate breakdown there are two oxido-reductions typical of glycolysis and fermentation, one of them involving the dismutation of hexosediphosphate via triosephosphate to phosphoglyceric acid and α -phosphoglycerol (Embden *et al.* 1933), and another between triosephosphate and pyruvate (aldehyde) to phosphoglyceric and lactic acid (alcohol) (Dische, 1935; Meyerhof & Kiessling, 1936). The latter has been much studied in yeast and in muscle as regards its mechanism (Meyerhof *et al.* 1937, 1938; Needham & Pillai, 1937; Needham & Lu, 1938). These investigations have greatly helped to solve the mystery of the role of inorganic phosphate in cell-free glycolysis and fermentation. It has been found that the last-named oxido-reduction of which cozymase is the catalyst involves uptake of inorganic phosphate by the adenylic system in the following manner. For every mol. of cozymase undergoing reduction to dihydrocozymase in the course of the reaction (oxidation of triosephosphate to phosphoglycerate), 1 mol. of phosphate is esterified by $MA \rightarrow ATP$. However, the oxidation of reduced cozymase by pyruvic acid (aldehyde) is not coupled with phosphorylation. Coupling of oxido-reductions with phosphorylations in red cells was shown by Dische (1936*a, b*), in yeast by Ostern *et al.* (1938), in bacteria by Lipmann (1939).

(b) *Other oxido-reductions.* In a vast number of enzymes (of animal, plant, yeast, bacterial origin) cozymase has been shown to be the specific hydrogen transferring coenzyme, for example for the lactic, malic, β -hydroxybutyric and glutamic dehydrogenases (skeletal muscle), alcohol dehydrogenase (liver, yeast, bacteria), aldehyde mutase (liver, kidney) and others. Dewan & Green (1937) have succeeded in linking various pairs of dehydrogenases by means of cozymase (see also Green *et al.*, 1937).

Warburg's cozymine acts in the hexosemonophosphate dehydrogenase (red cell, yeast) and also in the glucose (liver), glutamic (yeast, bacteria), phosphohexonic (yeast) and ribosephosphate (yeast) dehydrogenases.

The flavin-adenine dinucleotide acts as coenzyme for the *d*-amino-acid oxidase (kidney, liver) and also for the xanthine oxidase (milk, liver). However, its function seems to be also the formation of flavoproteins which catalyse the oxidation of dihydropyridine nucleotides.

IV. SUMMARY

Adenine derivatives represent a group of substances of considerable biological interest. Our knowledge of their occurrence in nature, properties and role is derived from both chemical and biological investigations.

With regard to chemical structure, they fall into three groups of different complexity: (1) the comparatively simple molecules adenosine, adenylic acid, adenylyl pyrophosphoric acid, (2) the intermediate compounds di-adenosine-pentaphosphoric and di-adenosine-tetraphosphoric acid, and (3) the dinucleotides cozymase, Warburg's coenzyme, amino-acid oxidase coenzyme, which apart from adenine possess yet another base (pyridine, alloxazine).

Adenosine does not occur in free state in nature. It can be obtained by hydrolysis from yeast nucleic acid. Adenosine is a substance of potent physiological activity, especially on the heart. Deamination of adenosine to inosine (e.g. by enzymes present in many tissues and body fluids) renders this substance physiologically inactive.

Adenylic acid (adenine nucleotide) is a general cell constituent. With regard to chemical structure we distinguish between muscle and yeast adenylic acid, the phosphoric group in the former being attached to C_5 , in the latter to C_3 , of the ribose molecule. Accordingly these two substances differ in several aspects, both chemical and biological. It is the muscle adenylic structure which is met with in the rest of the higher adenine derivatives.

Adenylic acid occurs in tissues and cells in the form of adenylyl pyrophosphoric acid and only seldom in free state (heart muscle). The pyrophosphoric group is easily split off, enzymically or by acid hydrolysis. A method is described for quantitative determination of adenylyl pyrophosphoric and adenylic acid in biological material.

In addition to adenylic and adenylyl pyrophosphoric acid there exist other polyphosphoric adenine derivatives, such as the di-adenosine-pentaphosphoric acid in heart muscle and the di-adenosine-tetraphosphoric acid in yeast.

Cozymase, diphosphopyridine nucleotide, originally called "coenzyme of alcoholic fermentation", is a substance of wide distribution in nature. Owing to the presence of the pyridine ring (nicotinic acid amide) in its molecule, it can undergo reversible oxidation and reduction: cozymase \rightleftharpoons dihydrocozymase. In the enzymic oxido-reduction systems (dehydrogenases) where it acts as coenzyme it plays the part of a hydrogen carrier, transferring the hydrogen from the substrate to a hydrogen acceptor such as flavoprotein.

Warburg's coenzyme, triphosphopyridine nucleotide, was first isolated from red blood cells and later found to occur in many other cells. Structurally it is closely related to cozymase, but it possesses one more phosphoric acid group than cozymase. The mechanism of its action as hydrogen carrier in enzyme systems is identical to that of cozymase, but in purified enzyme preparations these two coenzymes cannot replace each other in spite of their close similarity. There is some evidence, however, that cozymase and Warburg's coenzyme are to a certain degree interconvertible under the influence of cell extracts.

Alloxazine-adenine nucleotide is a substance of ubiquitous presence in biological material. Originally isolated from heart muscle and kidney as the coenzyme of the amino-acid oxidase, it was soon found to be essential for other enzymic proteins as well (flavoprotein, xanthine oxidase). Its role as hydrogen carrier is due to the reversible oxidation and reduction of the alloxazine ring.

The function of the adenine derivatives in biological processes, e.g. phosphate transfer by the adenylic system and hydrogen transfer by cozymase, is discussed in muscle glycolysis and alcoholic fermentation. Coupling is shown to occur between the phosphorylations and oxido-reductions in some of the intermediary stages of glycolysis and fermentation.

Enzyme systems are quoted in which the two pyridine dinucleotides and the alloxazine dinucleotide act as coenzymes.

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STABLE ISOTOPES AS INDICATORS IN BIOLOGY

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(Received 4 March 1939)

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I. INTRODUCTION

"THE same algebraic sum of positive and negative charges in the nucleus when the arithmetical sum is different gives what I call 'isotopes' or 'isotopic elements' because they occupy the same place in the periodic table. They are chemically identical, and save only as regards the relatively few physical properties which depend upon atomic mass directly, physically identical also."

According to this classical definition of Soddy, elements are associated exclusively with atomic number and may be defined as substances with definite chemical and spectroscopic properties, which may or may not be mixtures of isotopes with different atomic weights. If, in a molecule, the proportion of one of the less abundant isotopes of a particular element is increased, the chemical properties of the molecule should not change and it should not be chemically distinguishable from its natural analogue. From the biological point of view one would not expect the organism to be capable of differentiating between such isotopically enriched molecules and the normal ones. Actually this is not strictly the case, as will be seen below, water, in

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which the proportion of the heavy isotope of hydrogen has been enormously increased, has marked biological effects. The heavy isotope of hydrogen, deuterium, is exceptional, however, in that its atomic weight is double that of the naturally more abundant isotope, protium, and the biological distinction is made only when protium has been to a great extent replaced by deuterium.

Although the isotopes of the same element cannot be chemically or biologically differentiated there are physical methods by which they can be distinguished with relative ease and considerable accuracy. It is now obvious that isotopes can be used as indicators in a study of the chemical or biological fate of their more naturally abundant analogues. Chemically the value of such indicators lies in the fact that while they do not alter the course or nature of the reactions in which they are involved, they can be detected after the reaction has taken place; and in general they can be determined with much greater accuracy and convenience than their natural analogues. Biologically the value of the isotopes lies in the fact that when substances of biological interest are enriched with regard to a particular isotope they are still entirely physiological. Generally it has been the practice, in order to test the conversion of a substance *A* into a substance *B*, or to study the transport of *A*, to supply it to the organism in an amount far above that required physiologically. An excess of substance *B*, or of *A* in some particular organ, has then been taken as indication or proof of the conversion of *A* into *B*, or of its transport. The disadvantages of using a physiologically excessive amount of a metabolite are obvious. Moreover, the appearance of an excessive amount of *B* in such cases does not necessarily imply its origin from *A*. The excessive amount of *A* might have induced the formation of *B* from some other source, as is the case in the appearance of cholesterol after excessive fat feeding. If an element in *A* were enriched with respect to one of its isotopes this "labelled" substance could be fed in physiological amounts and its conversion studied by following the movements of the isotope. It would even be possible to feed the labelled compound in amounts lower than the physiological requirements and still to follow its conversion or transport. The use of isotopically-labelled substances thus presents a method whereby metabolism can be studied under physiologically normal equilibrium conditions.

The use of isotopes as indicators has been known now for several years. A natural radioactive indicator was first used in 1912 by von Hevesy & Paneth (1913) as an indicator in analytical work. Ten years later Th B was used in biological work when von Hevesy (1923) studied the transport of lead in plants. A more extensive use of radioactive isotopes, and the inclusion of isotopes other than the natural ones, was made possible when it was discovered by Curie & Joliot (1934) that radioactivity could be induced artificially by bombardment with α -rays from radium. Lawrence's perfection of the cyclotron has further simplified the preparation of radioactive isotopes. Radioactive lead, sodium, potassium, sulphur and phosphorus have now been applied to biological problems; of these the last, P^{32} , has been far the most used. von Hevesy (1938) has already published a comprehensive review of the biological application of radioactive isotopes, and the present review is accordingly restricted to a discussion of the stable isotopes.

The stable isotopes are of course not artificially prepared, but obtained by concentration. Their application to biological problems was made possible by the discovery of deuterium by Urey (Urey *et al.* 1932). Urey has since developed methods for the concentration of many of the stable isotopes, including hydrogen, nitrogen, oxygen, carbon and sulphur. Of these, deuterium is now available commercially; pure deuterium oxide, or "heavy water", is obtained as a by-product in the electrolytic production of hydrogen.

The application of radioactive isotopes as indicators does not differ in principle from the application of stable isotopes, though the methods for the determination differ considerably. The radioactive isotopes can be determined with far greater ease than the stable. The former can be accurately determined by means of a Geiger counter, while the latter, with the exception of the isotopes of hydrogen and oxygen, require a mass spectrometer for their analysis. In general, too, samples containing radioactive isotopes do not require such rigorous purification as those containing stable isotopes. Thus, while both the radioactive and the stable isotopes of sulphur are available, it would be far more convenient to use the former. Although it is possible to obtain the radioactive isotopes of almost all the elements, their application as indicators is restricted to those whose half-lifetime is sufficiently long. The half-lifetime of radioactive carbon is 21.5 min.; this is obviously not sufficiently long to permit of its incorporation into a suitable molecule and of subsequent biological experiments. Here the stable isotope of carbon would be more suitable for use as an indicator.

Deuterium has been extensively used in a large variety of biological experiments, and experiments on the biological application of the heavy isotope of nitrogen have recently been reported.

II. DEUTERIUM

1. *Biological effects*

Of all the isotopes, deuterium is the most different from its natural analogue; the atomic weight is doubled, the density of D_2O is 1.1074, its melting-point is $3.82^\circ C.$, and its boiling-point $1.42^\circ C.$ higher than those of H_2O , its viscosity is increased by about 25 %, and its solvent power is decreased—in the case of NaCl by as much as 15 %. It is reasonable to suspect, therefore, that D_2O should have appreciable biological effects. While the literature is confusing, and sometimes contradictory (partly because of the earlier use of impure heavy water), it seems that the biological effects of low concentrations are negligible. Higher concentrations of D_2O , from about 25 %, often have marked inhibitory or lethal effects. This subject has been reviewed by Meyer (1936) and by Barbour (1937). The effect of D_2O on enzyme action has also been studied. It appears that high concentrations can sometimes inhibit and sometimes activate enzymes. For a review of this aspect of the effects of D_2O see Bonhoeffer (1936).

The natural abundance of deuterium in water from numerous diverse sources is 0.02 %, and this ratio is also invariably observed in organic compounds and in biological material (see, for example, Dole, 1936). It appears that the organism is

unable to distinguish between deuterium and protium when the former is in low concentration. It is interesting, however, that the organism is capable of carrying out an appreciable fractionation of the isotopes of potassium; Lasnitzki & Brewer (1938) have observed an increase of K^{41} in bone marrow and a decrease in tumours. When deuterium is used as an indicator in biological studies it is almost always used in very low concentrations, and it is safe therefore to assume that the biological effects under these conditions are negligible.

2. Principles of application

The types of experiment in which deuterium has been used as an indicator fall into two main classes. In the first of these the metabolism of a compound which has been labelled with deuterium is studied. The label is attached by substituting deuterium for carbon-bound hydrogen in the molecule and the compound is supplied to a suitable animal. The transport of this compound to a particular organ, or its conversion to other substances, can then be studied by determining the deuterium content of the various organs, or of pure compounds isolated from the biological system. If a compound *A* is labelled with deuterium and a compound *B* is subsequently isolated and found to contain a suitable amount of deuterium, we have reasonable proof that *B* was derived from *A*. Obviously *B* must not be contaminated with traces of *A*, or with breakdown products of *A*. The deuterium content of *B* must also be appreciably higher than that of the body fluids of the experimental animal, since otherwise the deuterium could have been introduced into *B* by its synthesis in the heavy-water medium from sources other than *A*. D_2O in the body fluids can be the result of the breakdown of *A* by oxidation or by other processes. In this type of experiment only positive results are of any value; the absence of deuterium in *B* by no means proves that it was not derived from *A*, since the label might well have been lost during the conversion process.

The second type of experiment involves increasing the heavy-water content of the medium in which biological reactions take place. With animals this is done by injecting them with concentrated heavy water to raise the level of heavy water in their body fluids. This level is maintained for various periods by giving the animals water with a suitable D_2O content to drink. Organisms like yeast can be grown in heavy water. After a suitable time substances are isolated from the organism and their content of carbon-bound deuterium determined. This is taken as a measure of the rate and amount of synthesis of the isolated substance. It must not, however, be assumed that all reactions taking place in heavy water will involve the introduction of carbon-bound deuterium in the end-products. Fredenhagen & Bonhoeffer (1938) have shown that when the Canizarro reaction is carried out in heavy water no stable deuterium is introduced into the alcohol or into the acid; similar results were found with the base-catalysed mutarotation of *d*-glucose, the rearrangement of glucose to fructose, the formation of hexose from glyceraldehyde and in the aldol condensation (Bonhoeffer & Walters, 1938).

Deuterium has been introduced into organic compounds in a number of ways. The simplest method is the reduction of an unsaturated compound with deuterium

gas. Thus stearic acid-6, 7, 9, 10- d_4 has been obtained by the deuteration of linoleic acid (Schoenheimer & Rittenberg, 1935*a*), and butyric acid- α , β - d_2 and caproic acid- α , β , γ , δ - d_4 by the deuteration of crotonic and sorbic acids (Rittenberg *et al.* 1937). This method is, of course, only applicable when the unsaturated compound corresponding to the desired deuterocompound is available. The unsaturated fatty acids above C_8 and below C_{18} are either not available at all or are not easily purified. van Heyningen *et al.* (1938) have developed a method whereby saturated fatty acids of almost any desired deuterium content can be prepared. This method is based on the platinum-catalysed exchange of hydrogen between saturated fatty acids and water. It was hoped that this method could be applied to other compounds, such as cholesterol, but so far it has been successful only with saturated fatty acids and saturated hydrocarbons. Patterson & du Vigneaud (1938) have prepared tetra-deuterohomocystine and dideuteromethionine by synthesis, starting from CaC_2 and D_2O . Other synthetic methods have also been applied; thus, for instance, Halford & Anderson (1936) have prepared malonic- d_3 -acid- d_2 by the interaction of carbon suboxide and D_2O , $C_3O_2 + 2D_2O = CD_2(COOD)_2$, and succinic- d_4 -acid- d_2 by reduction of potassium acetylene dicarboxylate by means of sodium amalgam and D_2O , $C_2(COO)_2^- + 4Na + 4D_2O = (CD_2COO)_2^- + 4Na + 4OD^-$, followed by acidification with DBr in D_2O , $(CD_2COO)_2^- + 2D^+ = (CD_2COOD)_2$.

Sometimes advantage has been taken of biological synthesis, as in the preparation of an unsaturated deuterio-fatty acid by the biological desaturation of a saturated deuterio-fatty acid (Rittenberg & Schoenheimer, 1937*a*). Foster *et al.* (1938) have isolated a number of deuterioamino acids from mice which had been kept for some time with a raised level of D_2O in their body fluids.

The determination of deuterium in organic compounds is a comparatively simple matter. The principle is as follows: the material is well dried and burned in a stream of dry oxygen, and the water of combustion, which now contains all the deuterium, is collected in a trap cooled in solid carbon dioxide. This water is then rigorously purified and its deuterium content determined. This is generally done by accurately determining its density. Since the density of pure D_2O is about 10% higher than that of H_2O it follows that the deuterium content of mixtures can be calculated from their densities. The three main methods which have been used for density determination are the pycnometer method, the float method, and the falling-drop method; for the pycnometer method see Barrett *et al.* (1938). It requires a sample of about 300 mg. and is accurate to 0.03% D_2O . The float method of Richards & Shipley (1912) is more sensitive and is particularly useful when only small samples of low deuterium content are available (see Rittenberg & Schoenheimer, 1935). It can be used for concentrations of deuterium below 0.03 atom % (i.e. 0.03 atom of deuterium per 100 total hydrogen atoms). It is not, however, a very convenient routine method. Keston *et al.* (1939) have published a detailed account of the routine method which is used in the New York laboratory for the determination of deuterium. Here a modification of the convenient falling-drop method originally described by Barbour & Hamilton (1924) is used. By this means the density of water can be determined with an accuracy of 1 part per million.

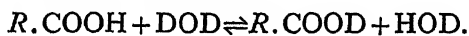
The deuterium content of a heavy-water sample can also be calculated from a comparison of its refractive index with that of ordinary water by the method of Crist *et al.* (1933). The difference in refractive index of H_2O and D_2O is 0.00462 for the sodium D line at 20° C. The determinations, which are carried out in a Zeiss interferometer, give an accuracy of about 0.02 atom %. About 400 mg. of water are required.

For an account of other methods used in the determination of deuterium see Harteck (1938).

3. The stability of the carbon-hydrogen bond

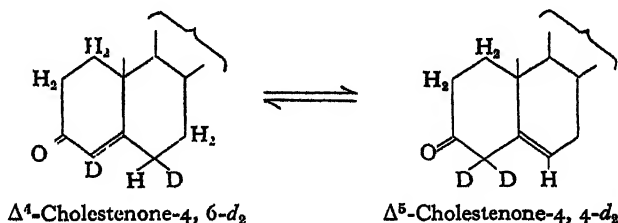
In practically all the experiments in which deuterium has been used as an indicator the interest has been not so much in the hydrogen itself as in the molecule to which it is attached. It is therefore important that the stability with which the label is attached to the molecule should be known.

It is already well known that the hydrogen not directly attached to carbon, but to oxygen or nitrogen, as in $-OH$, $-COOH$ and $-NHR$ groups is extremely labile (for references see Kharasch *et al.* 1937). Such hydrogen will exchange immediately with the hydrogen of water when the compound is brought into aqueous solution. Thus, if a compound $RCOOH$ is placed in heavy water the following reaction will immediately take place:



This type of action is completely reversible.

When the hydrogen is attached to a carbon atom adjacent to a carbonyl group it is also exchangeable, this being the result of enolization which causes the hydrogen temporarily to be attached to oxygen. A double-bond rearrangement might also render a hydrogen atom exchangeable by causing it to be moved to a labile position. This is the case, for instance, with Δ^4 -cholestenone-4, 6- d_2 . Here the following rearrangement takes place in alkali:



All the deuterium is now in a position to become exchangeable by enolization (Anchel & Schoenheimer, 1938).

In the aldehyde group $R.C \begin{smallmatrix} \diagup H \\ = O \end{smallmatrix}$ the hydrogen which is attached to the same carbon atom as the oxygen does not seem to be exchangeable; nor does the carbon-bound hydrogen of the alcoholic group $R_2.CH-OH$. The hydrogen attached to the carbon atom in the α -position to a carboxyl group seems on the whole to be

stable, though in some cases it may be "semi-stable". In malonic acid, as might be expected from the reactivity of this position, the methylene hydrogen is rapidly exchangeable. The methylene hydrogen of succinic acid, on the other hand, appears to be completely stable in spite of its biological reactivity. van Heyningen *et al.* (1938) have shown that the deuterium which is introduced into saturated fatty acids by treatment with D_2SO_4 is attached to the α -carbon atom only.

Naturally when deuterium is attached in one of the easily exchangeable positions described above it is of no use as an indicator and is therefore always removed before analysis of a sample. This is done by repeatedly dissolving the compound in ordinary water and reprecipitating, or by repeated crystallization from water.

An apology is due here for the use of the terms "stable" and "labile" in connexion with hydrogen. They are used for convenience in describing the bond with which hydrogen is attached to other atoms, and do not refer to the hydrogen itself, which is stable in the sense that it does not disintegrate like the radioactive elements.

Stekol & Hamill (1937) have treated cystine at $110^\circ C.$ for 4 days, and the hydrochlorides of histidine, lysine and arginine at $150^\circ C.$ for 42 hr. with dilute heavy water in the presence of dilute acid. Under these conditions a small amount of deuterium entered the amino-acid molecules. The deuterium was carbon bound and could not be washed out with ordinary water at room temperature. The cystine was racemized. Rittenberg *et al.* (1938) treated glycine, proline, phenylalanine, tyrosine, cystine, glutamic acid and lysine in a similar manner, but at $108.6^\circ C.$ Under these conditions they were unable to introduce deuterium into lysine, but the result with cystine was confirmed. They also found deuterium equivalent to an exchange of less than one atom per molecule in glycine, tyrosine and glutamic acid. That the carbon-bound hydrogen of glycine is semi-labile had already been shown by Günther & Bonhoeffer (1937). In the case of cystine the deuterium is probably introduced at the α -carbon atom during racemization. During such a process the α -hydrogen is probably in a fairly labile state and ready to exchange since Erlenneyer *et al.* (1937) have observed that the racemization of *l*-menthyl phenylbromoacetate in D_2O is accompanied by an uptake of deuterium. Rittenberg *et al.* were able to show that the uptake of deuterium into tyrosine takes place in the aromatic nucleus in the *o*-positions to the hydroxyl group. On bromination in these positions the deuterium was lost. As will be seen below, such a finding is predictable. They also suggest that the deuterium uptake in the case of glutamic acid might be due to the equilibrium of this compound with pyrrolidonecarboxylic acid in acid solution.

If, in the course of the biological synthesis of proteins and amino acids in a heavy-water medium, deuterium is introduced into such semi-labile positions it will be lost again during the process of isolation which involves boiling with dilute acid. No conclusions could, of course, be based on such negative results; on the other hand, such semi-labile hydrogen cannot lead to a positive finding of deuterium. It has always been the practice of Schoenheimer and his collaborators to treat all samples with boiling dilute acid or alkali before analysis in order to remove semi-

labile deuterium, and conclusions have only been based on the content of the more stably bound isotope.

Under certain drastic conditions the hydrogen in almost any position in an organic molecule can be induced to exchange with the hydrogen of its surroundings. Thus Horrex & Polanyi (1935-6) have found that the exchange of hydrogen between saturated hydrocarbons and water can be catalysed by platinum at elevated temperatures, and, as has been seen above, van Heyningen *et al.* have taken advantage of this exchange in the preparation of a series of deuterio-saturated fatty acids. In this case equilibrium was not reached after shaking the fatty acids with heavy water and catalyst for more than a week at about 130° C. Geib (1937) has studied the velocity of the exchange of hydrogen between resorcinol and pyrogallol and heavy water which is catalysed by acid at high temperatures. With resorcinol only five of the six hydrogen atoms exchange at 230° C. The one which does not exchange is probably the nuclear hydrogen in the *m*-positions to the two hydroxyl groups. The hydrogen at (2) exchanges six times as slowly as those at (4) and (6). In pyrogallol all the hydrogen atoms were found to exchange eventually, but that at position (5) exchanges 2000 times more slowly than those at positions (4) and (6). Geib ascribes the differences in the exchange velocities in these positions to differences in their activation energies, and concludes that there must be a close relationship between reactivity for exchange and reactivity for other ionic reactions. Koizumi & Titani (1938) found similar results for the exchange of hydrogen between nitrophenol and water. They find that two nuclear hydrogen atoms exchange in *o*- and *p*-nitrophenol and three in the case of *m*-nitrophenol. Ingold *et al.* (1936*a*) found that deuterium can be introduced into aromatic compounds by treatment with D₂SO₄, and that the amount introduced depends on the nature of substituents in the aromatic nucleus. They conclude that the deuteration of substitution products of benzene follows the known rules for orientation and velocity in electrophilic aromatic substitution. Thus it would seem that the possibilities for exchange in aromatic compounds are predictable. Matters are not so simple, however, for straight chain and cyclic aliphatic compounds. Here Ingold *et al.* (1936*b*) find that the deuteration by D₂SO₄ does not stop rapidly in relation to a limited number of positions in the molecule, but that the uptake differs with various hydrocarbons and apparently is arbitrary. The deuteration is extremely slow in cyclohexane, for instance, and rapid and extensive in methylcyclohexane.

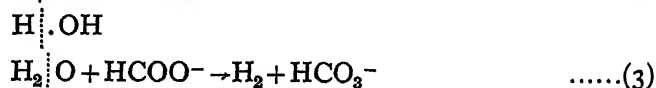
From what has been discussed above it is evident that our present knowledge of organic chemistry does not always allow us to predict with certainty the possibility of the isotopic indicator being lost from the molecule. The exchange conditions which have been discussed do not, of course, exist in the organism, and it is essential, whenever possible, to test the possibilities for exchange under biological conditions. As has been mentioned before, Schoenheimer and his collaborators always remove the deuterium that might be in semi-labile positions in compounds after they have been isolated from a biological system. There is always the possibility, however, of the existence of an enzyme which is capable of catalysing the exchange of carbon-bound and apparently stable deuterium. Some experiments have been carried out

in an attempt to test this possibility. For this purpose Schoenheimer & Rittenberg (1936*b*) have used the developing hen's egg. A physiological saline solution in D_2O was injected into a number of eggs on the first day of development. The embryos developed normally, and after 20 days the chicks were completely developed. From these, fatty acids were isolated and found to contain no deuterium. Schoenheimer & Rittenberg conclude that the developing egg contains no enzyme capable of catalysing the exchange of the carbon-bound hydrogen of fatty acids, since otherwise these would have contained deuterium. They state that the developing chick, as far as is known, contains all the enzymes present in adult animals and therefore feel justified in postulating that mice also do not contain such an enzyme. They found similar results with cholesterol (Rittenberg & Schoenheimer, 1937*b*). The fact that of fourteen amino acids isolated from mice whose body fluids had been enriched with D_2O only lysine was free of deuterium is also taken as evidence of the absence of a hydrogen-labilizing enzyme. As lysine is known to be an indispensable amino acid it is not surprising that no chemical reactions took place that might have caused it to take up deuterium; but if there had been hydrogen-labilizing enzymes in the mice it should have become "heavy", Rittenberg & Schoenheimer conclude that since the hydrogen of the methylene groups of lysine is not exchangeable *in vivo*, the possibility that the hydrogen of the methylene groups of other compounds is also not exchangeable under these conditions is increased.

Barrett *et al.* (1938) have also carried out some experiments designed to test the biological stability of the carbon-bound hydrogen of fatty acids. Mice were maintained for some time on a diet containing deuterio-fat, and the deuterium content of the glycogen and protein subsequently isolated from these animals was determined. In either case this was found to be zero, and it was concluded therefore that there is no enzyme system capable of catalysing the exchange of hydrogen between fatty acids on the one hand and glycogen and protein on the other.

Stekol & Hamill (1937) claim to have shown that deuterium is introduced into carbon-bound positions in tyrosine during the tryptic digestion of casein in heavy water. This important claim could not be confirmed by Foster *et al.* (1938).

The possibility of a hydrogen labilizing must not, however, be dismissed too readily. The first experiments in which deuterium was used as an indicator in enzyme studies were those of Farkas *et al.* (1934). The enzyme hydrogenlyase of *Bacterium coli* catalyses the production of CO_2 and H_2 from formic acid in an aqueous medium (Stephenson & Stickland, 1932), and the question of the origin of the hydrogen arises. It might be derived entirely from the water, or from the formic acid and water. (It cannot be derived from formic acid alone because of the rapid exchange of the carboxyl hydrogen.) The following mechanisms are possible:



The enzyme-catalysed reaction was carried out in a heavy-water medium, and it was thought that the isotope content of the evolved hydrogen would reveal its source. Unfortunately, it was found that *Bact. coli*, like platinum black, also catalyses the exchange of hydrogen between molecular hydrogen and water. Thus it is obvious that deuterium cannot be used in determining the source of hydrogen produced by formic hydrogenlyase. The results of these experiments were too optimistically interpreted by Krebs (1937) who concludes that Farkas *et al.* have shown that mechanisms (1) and (2) do not hold.

4. Metabolism of water

von Hevesy & Hofer (1934*a*) have determined the retention time of water in human beings. They drank up to 2 l. of 0.46% D_2O (4600 parts per million), and, by estimating the density of the urine water, were able to determine how much of the ingested water was excreted within a given time in the urine. Thus, for instance, the occurrence of 10 parts of D_2O in a million parts of urine water would account for 1/500th of the ingested water. Heavy water first began to appear in the urine after 26 min., when 1 ml. of the ingested 2 l. was excreted. The rest of the water was very slowly excreted, and after 10 days half of it had appeared in the urine. From this the average retention time of a molecule of water was calculated to be $10/\ln 2 = 14$ days. The ingested water is diluted by the body water, and from the deuterium content of the urine von Hevesy & Hofer were able to calculate the water content of a human being. This they found to be 43 l. for a man weighing 69 kg. The value of 62% thus obtained is in good agreement with previous determinations. Heavy water is a superior indicator to methylene blue in experiments of this nature, since the latter becomes detectable in the urine only after 2 hr. and is not found any more after 43 hr.

von Hevesy & Hofer (1934*b*) have also investigated the exchange between the water of the fish and that surrounding it. They placed a goldfish in dilute heavy water and after 1-5 hr. they measured the decrease in the deuterium content of this water. From the values thus obtained the amount of exchange was calculated and it was found that after 4 hr. the water of the fish had exchanged completely with the water of its surroundings. The rate of this exchange is inversely proportional to the size of the fish and is appreciably slower when the fish is dead.

Dilute heavy water is ideally suited to a study of the permeability of various membranes to water. The difference in the radii of the HOH and DOD molecules is only 0.1%, and the difference between HOH and HOD, which is the form in which deuterium occurs in dilute heavy water, is still smaller. von Hevesy *et al.* (1935), using 2-5% D_2O , have found that the permeability of frog skin is 400 days at 0° C., as compared with 0.07 day for collodion membranes. (Permeability is defined as the time in which 1 g.mol. of water passes through 1 sq.cm. of membrane with a concentration gradient of 1.) The question of whether or not the frog skin is equally permeable in both directions was answered when they found that there was no difference; nor did they find any difference between the intact and isolated skin.

The permeability was found to increase with increasing temperature—at 22° C. it is 130 days—but this is ascribed to the decreasing viscosity of the water. No direct influence of the nervous system was observed; after the nervus ischiadicus had been severed an increase in permeability in both directions was observed, but this is ascribed to the increased blood circulation since normal permeability was re-established after a few days.

5. *Metabolism of acetic acid*

Sonderhoff & Thomas (1937) have investigated the metabolism of trideutero-acetic acid by yeast. Sodium trideuteroacetate containing 86 atoms % of deuterium was prepared by the method of Wilson (1935) and incubated with yeast; sodium succinate was subsequently isolated and found to contain 40.6 atoms % deuterium. From this it was concluded that the acetic acid had undergone dehydrogenation. While it is safe to conclude that the succinic acid isolated was derived mainly from the acetic acid it is by no means certain that the formation of succinic acid was brought about by simple dehydrogenation. If this had been the case the succinic acid should have had the same deuterium content as the acetic acid; actually the deuterium content decreased by about half. That this loss was not due to an exchange of hydrogen in the acetic acid is shown by the fact that when unused acetic acid was isolated its deuterium content was found to be unchanged.

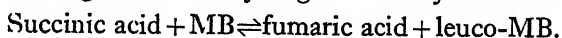
Sonderhoff & Thomas explain the low deuterium content of the succinic acid by assuming that it is in an "activated" form in which the hydrogen is labile. In previous work by Wieland & Sonderhoff (1932) it was claimed that succinic acid newly formed from acetic acid was more rapidly broken down than succinic acid added to yeast. The hypothesis of an "active" form of a substance is always unsatisfactory, and the loss of deuterium from succinic acid could equally well be due to chemical reactions taking place during its formation from acetic acid by a process more complicated than simple dehydrogenation. Alternatively, the deuterium in the succinate could have been diluted by the concurrent formation of "light" succinate from other sources, such as the glutamic acid of yeast protein. Sonderhoff & Thomas claim that their experiments have ruled out such a possibility, but this claim is not justifiable since their technique has not shown that the succinate was derived exclusively from the acetate. The possibility of bacterial infection having altered the course of events cannot be excluded, since their description of their experiments gives no assurance that they were carried out with sterile precautions.

Citric acid which was isolated from yeast after it had grown on barium tri-deuteroacetate contained 55.8 atoms % of deuterium. Here again the deuterium values, although they do indicate the origin of the citric acid, can give no reliable information as to its mode of formation.

More interesting results were obtained from the fat isolated from the yeast after it had grown on trideuteroacetate. The total fats contained about 23 atoms % of deuterium, and the unsaponifiable portion of the fats contained 31 atoms % of deuterium. Since this latter value must be higher than that of the fatty acids

Sonderhoff & Thomas conclude that the steroids of yeast cannot be derived from fatty acids, but are probably derived directly from acetic acid.

Erlenmeyer *et al.* (1936) have studied the dehydrogenation of α , β - d_2 -succinic acid by methylene blue in the presence of succinic dehydrogenase. No quantitative conclusions can be drawn from the deuterium values, since the deuterium of the deuteriosuccinate exchanged with the hydrogen of methylene blue in the equilibrium



6. Metabolism of fatty acids

Probably the most intensive application of deuterium to biological work has been in the study of fat metabolism. Schoenheimer & Rittenberg (1935*b*) were able to show that the bulk of ingested fatty acids are deposited before being utilized, even under conditions of starvation. They fed mice for 2–10 days on diets consisting mainly of carbohydrate and containing 20, 4 and 1 % of fat in the form of partially deuterated linseed oil containing 5.74 atoms % of deuterium. At the end of the feeding periods the fat of the fat depots (this was taken to be the fat of the carcass after removal of the internal organs), the fat of the internal organs and the body fluids were analysed for deuterium. These values showed that the bulk of the ingested fat was in the depots. The amount of ingested fat in the depots is given by the expression $x/y \times z$ mg., where x is the deuterium content of the depot fat, y the deuterium content of the ingested fat, and z the weight in mg. of the depot fat. The amount of absorbed fat is obtained by subtracting the amount of combined fat of the uncaten food, the alimentary tract and the faeces from the amount of fat supplied. In the experiment in which mice were maintained on a 1 % fat diet, 47 % of the ingested fat was found in the depots, and 20 % as water in the body fluids, while a small amount was found in the internal organs.

Cavanagh & Raper (1936) fed deuterated linseed oil containing 4.9 atoms % of deuterium to rats for 7 days and found that the ingested fatty acids entered largely into the lipoids of the liver and kidney. They found 0.98 atom % deuterium in the liver lipoids, 0.97 atom % in the liver glycerides, 0.63 atom % in the kidney lipoids and 0.75 atom % in the adipose tissue fat, and no deuterium in the fat of the faeces, showing that the ingested fat was almost completely absorbed.

This investigation was extended (Cavanagh & Raper, 1939), and in the later experiments the animals were killed 6, 10 and 24 hr. after the deuterio-fat had been fed. Even after these short periods essentially the same results were observed. After 6 hr. there was a considerable amount of deuterium in the plasma glyceride (1.26 atoms %), liver glyceride (0.86 atom %) and liver lipoids (0.47 atom %). The liver glyceride value decreased to 0.30 atom % in 24 hr., and the liver lipid only to 0.42 atom %. The kidney and plasma lipid fractions were very low after 6 hr. and did not change after 24 hr., while only traces of deuterium were found in the brain glyceride and lipid after 6 and 24 hr.

Barrett *et al.* (1938) have used deuterium in a study of the source of liver fat. It is known that an accumulation of fat in the liver takes place when animals are

starved or fed on a high carbohydrate diet, when an extract of the anterior pituitary gland is administered, and when they are exposed to carbon tetrachloride vapours. In a study of the increase of liver fat on starvation, mice were put on a diet containing deuterated linseed oil for several days. After this period some of the mice were killed to serve as controls, while others were starved from 24 to 168 hr. In the control animals it was found that the deuterium content of the liver fat was higher than that of the depot fats; this was no doubt due to dilution with ordinary fat already present in the depots. In the starved animals the liver fat was found to have increased two to three times, while its deuterium content had dropped to approximately the same level as that of the depot fats, which had remained roughly constant. In spite of the drop in the deuterium content of the liver fat, it was still considerably higher than it would have been had the increase in the amount of fat been derived from a source free of deuterium, or from newly formed fat. Barrett *et al.* were therefore able to conclude that the increased liver fat was derived mainly from a deuterium-containing source of fat, namely, the fat depots. Thus in one experiment a group of animals was fed on a diet containing 20% deuterated linseed oil, with a deuterium content of 6.51 atoms %. After 14 days a control group was killed and another group was starved for 52 hr. In the control group the liver fat per mouse amounted to 37 mg., with a deuterium content of 3.67 atoms %, while the depot fat weighed 5.3 g. and had a deuterium content of 2.69 atoms %. In the starved animals the liver fat rose to 96 mg., with a deuterium content of 2.65 atoms %; the depot fat weighed 3.4 g. and contained 2.44 atoms % deuterium. If the increase from 37 to 96 mg. in the liver fat was entirely due to deuterium-free fat its deuterium content should have dropped to $37/96 \times 3.67 = 1.41$ atoms %; actually it dropped only to 2.65 atoms %, a value which is still above that of the depot fat. Therefore it is safe to assume that a considerable proportion of the increase in liver fat was derived from the depot fat.

The increase in liver fat resulting from an injection of an extract of the anterior pituitary gland of an ox and from exposure to carbon tetrachloride vapours was also traced to the depot fat by experiments similar to those described above.

When animals are kept on a high carbohydrate diet poor in lipotropic factors they also develop fatty livers. Mice were fed for an initial period on a diet containing deuterio-fat, after which some controls were killed while others were maintained for 52–168 hr. on a diet containing 91% carbohydrate and no fat. Here it was found that the deuterium content of the increased liver fat fell to a value well below that of the depot fat, which remained approximately constant. In one experiment the liver-fat content per mouse in the control group was found to be 37 mg. with a deuterium content of 3.67 atoms %. In the group which were then maintained for 7 days on a carbohydrate diet the liver fat increased to 90 mg. per mouse. If none of the liver fat had been utilized or transported away and if the increase had been derived entirely from a deuterium-free source, the deuterium should have been $37/90 \times 3.67 = 1.51$ atoms %; actually it was found to be even lower, 1.20 atoms %. Though this is a negative result as far as deuterium is concerned, it is, in view of the known stability of the carbon-bound hydrogen of fatty

acids, reasonable to assume that the increased liver fat was not derived from the fat depots. Barrett *et al.* infer that the new fat arises probably by synthesis from carbohydrate. While this is probable, it must be pointed out that such a synthesis has not been directly proved by the experiments.

The problem of the metabolism of the lower fatty acids is interesting. According to the theory of β -oxidation myristic, lauric, capric, caprylic, caproic and butyric acids should be intermediates in the combustion of stearic and palmitic acids. Actually fatty acids with a chain length of less than 16 carbon atoms occur only to a limited extent, in animal fat depots which always predominate in saturated and unsaturated C_{16} and C_{18} acids. In preliminary experiments (unpublished) van Heyningen has obtained results which indicate that under the same conditions about 50% of absorbed stearic and palmitic acids are deposited, while the C_{12} acid, lauric acid, is deposited to about half that extent.

It has been shown that even after extensive feeding butyric and caproic acids are not found in the depots (see Eckstein, 1929). The question therefore arises as to whether these lower fatty acids are immediately disposed of, by combustion for example, or converted into higher fatty acids. Eckstein, from the results of balance experiments, claims that the latter route is followed. Rittenberg *et al.* (1937) have used deuterium in a reinvestigation of this question. Deuterobutyric acid was prepared by the deuteration of ethyl crotonate and given by stomach tube to mice which were killed 8 hr. later. No deuterium was found in the depot fat, but sufficient was found in the body water to account for the complete combustion of the deuterobutyric acid, assuming, of course, that deuterium was introduced into the body water by oxidation of the acid, and not by some other process. Such a loss of deuterium from the deuterobutyric acid might occur during its hypothetical conversion to higher fatty acids, in the course of which intermediates, such as acetoacetic acid containing labile deuterium, might be formed. Working on the assumption that in the conversion of butyric acid to the higher fatty acids reactions such as reductions, hydrations and condensations must necessarily occur, Rittenberg *et al.* carried out the reverse experiment of artificially raising the heavy-water content of mice fed ordinary butyric acid. "If the synthesis of stearic acid or other higher fatty acids from butyric acid is carried out in a medium of heavy water, deuterium must be expected in the synthesized fatty acid, as no route of synthesis can be formulated in which none of these reactions is involved."

Mice were injected with enough concentrated heavy water to raise the deuterium content of their body fluids to about 3 atoms %; 30 min. later they were given ordinary butyric acid, and 6 hr. later they were killed. The fatty acids from the carcasses were found to contain a negligible amount of deuterium. From these results, and on the basis of the above-mentioned argument, it was concluded that an appreciable conversion of butyric acid into higher fatty acids could be excluded. Such an argument, excluding as it does the possibility of conversions taking place, the steps in which we are not yet able to formulate, is unfortunate. Nevertheless, the conclusion is probably still valid; the second experiment has shown that either the synthesis into higher fatty acids did not take place, or that it did take place

without the formation of intermediates containing labile deuterium. The appearance of all the deuterium in the body fluids in the first experiment excludes this second alternative. The butyric acid was of course not necessarily disposed of by combustion; the possibility of its conversion into substances other than the higher fatty acids with concurrent loss of deuterium has not been excluded. Similar experiments with similar results were carried out on deuterocaproic acid.

By direct experiments with deuterium the New York group were able to follow the desaturation (Schoenheimer & Rittenberg, 1936*a*) and saturation (Rittenberg & Schoenheimer, 1937*a*) of fatty acids in the organism. Experiments in which desaturation is followed by observing an increase in iodine number are not entirely satisfactory, since this might be due to synthesis of unsaturated fatty acids from non-fatty material. Schoenheimer & Rittenberg also believe that the appearance of unsaturated fatty acids in the lymph after ingestion of saturated fatty acids can be explained by the transport of unsaturated acids by lymph or blood from other organs to the intestinal system.

Mice were fed saturated deuterio-fatty acids and the deuterium-containing fatty acids of the carcasses were then separated into saturated and unsaturated fractions. The finding of deuterium in the unsaturated acids constitutes definite proof of their origin from the saturated acids. In one experiment, for example, mice were fed for 12 days on a diet containing 10% of fatty acids which had a zero iodine number and a deuterium content of 8.66 atoms %. 4.46 g. of total fatty acids containing 1.70 atoms % deuterium were isolated from the mice. Of this 1.61 g. were saturated fatty acids containing 2.58 atoms % deuterium, and 2.03 g. were unsaturated fatty acids containing 1.16 atoms % deuterium. The body water contained 0.21 atom % deuterium. In experiments of this nature it is obviously essential to ensure that the unsaturated acids are free from the deuterium-containing saturated acids. Saturated fatty acids are separated from the unsaturated by precipitating them as their lead salts, but this method is not entirely quantitative, and about 5–10% of the saturated acids remain in the mother liquor to contaminate the unsaturated acids. While they could not remove this residue of saturated fatty acids they were able to "wash out" the *deutero*-fatty acid by repeated addition of ordinary saturated fatty acid to the mother liquor and reprecipitation as the lead salt. Control experiments showed that the unsaturated acids could be completely freed of deutero-saturated acid. This method of "washing out" of isotopic with non-isotopic material had also been used with radioactive elements (see von Hevesy, 1938).

In a study of the biological hydrogenation of unsaturated fatty acid use was made of deutero-unsaturated fatty acids obtained biologically by means of the experiments just described. Deutero-unsaturated fatty acids prepared by partial deuteration of highly unsaturated fatty acids are not satisfactory, since an unphysiological mixture, probably containing acids with a *trans*-configuration, is obtained. In this study two experiments were carried out on single mice, and the results are not as clear-cut as those of the experiments on desaturation. Two mice were maintained in the same cage for 10 days, during which period they consumed 5.56 g. of unsaturated fatty acid ester containing 0.93 atom % deuterium. Here

there was no difficulty in freeing the saturated acids from the deuterium-containing unsaturated acids; they were precipitated as their lead salts and recrystallized from alcohol. The unsaturated acids were purified as before. The results of this experiment are given in Table I. It will be seen that the deuterium content of the

Table I. *Deuterium content of fatty acids and body fluids of mice*
(Atoms % deuterium)

Mouse	Unsaturated acids	Saturated acids	Body fluids
1	0.25	0.047	0.043
2	0.10	0.025	0.028

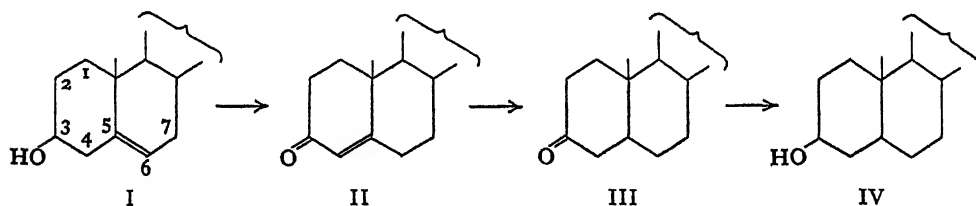
saturated acids is the same as that of the body fluids, and the obvious question arises as to whether these saturated deuterio-acids could not have been synthesized from a non-fatty source in the heavy-water medium. Schoenheimer & Rittenberg (1936*b*), however, had already shown that when the deuterium content of the body fluids is raised the deuterium content of the fatty acids is only one-quarter to one-third of that of the body fluids when equilibrium is reached (in 6–8 days, with a high level of deuterium in the body fluids from the first day; in these experiments the deuterium level rose from 0 to 0.043 and 0.028 atom % in 10 days). Rittenberg & Schoenheimer conclude therefore that at least two-thirds and possibly more of the saturated deuterio-acids originated from the ingested unsaturated acids. The ratio of deuterium in the saturated acids to that in the unsaturated acids is 0.19 in one experiment and 0.25 in the other; from these figures they infer that approximately 20% of the saturated fatty acids were derived from the unsaturated fatty acids. Difficulties in obtaining unsaturated deuterio-acids do not allow a more statistical treatment in these calculations.

According to the theory of β -oxidation the organism should be able to convert the C_{18} fatty acid, stearic acid, into the C_{16} acid, palmitic acid. Schoenheimer & Rittenberg (1937) were able to show that such a conversion does take place. Ten mice were fed for 5 days on a diet containing 8% ethyl deuterostearate (7.00 atoms % deuterium); they were then killed and the body fluids were found to contain 0.20 atom % deuterium and the total fatty acids 0.72 atom %. The saturated fatty acids were isolated from the total fatty acids, and from them in turn was isolated the palmitic acid fraction containing 0.32 atom % deuterium. This value, which is well above that of the body fluids, shows that the deuterostearic acid was converted into deuteropalmitic acid. The saturated fatty acid was fractionated by fractional distillation of their methyl esters under reduced pressure at temperatures of over 150° C. in a special still. It was important to ensure that an exchange of deuterium between the various fatty acid fractions does not occur under these conditions. Deuterostearic acid and ordinary palmitic acid were accordingly mixed and distilled under the same conditions as the carcass fatty acids; no deuterium was found in the palmitic acid. In the distillation of the carcass fatty acids it was also important to

ensure that the palmitic acid fraction was not contaminated with traces of deuterostearic acid. Such traces were washed out by mixing ordinary acid with the palmitic acid fraction and redistilling.

7. Metabolism of steroids

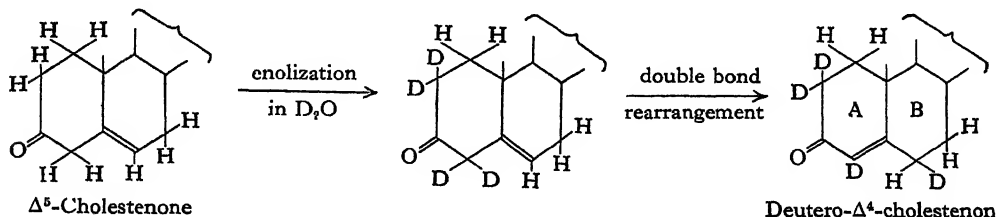
Schoenheimer & Rittenberg and their collaborators have used deuterium in a study of the excretion of coprosterol (Schoenheimer *et al.* 1935; Anchel & Schoenheimer, 1938). Cholesterol is usually excreted as coprosterol in the faeces, and various workers have shown the intestinal bacteria take part in this conversion (see Dam, 1934). Since coprosterol cannot be obtained directly from cholesterol, the reduction *in vitro* always leading to the formation of dihydrocholesterol, it was thought that the conversion is indirect, with the formation of intermediary compounds. Cholesterol (I) can be converted *in vitro* into coprosterol (IV), without the formation of any dihydrocholesterol, through cholestenone (II) and coprostanone (III):



Schoenheimer *et al.*, in balance experiments, found that ingested cholestenone causes an increased coprosterol output in the stools of a dog fed on a meat diet, but when dogs were put on a diet of dog biscuits the excess sterol in the stools was mainly cholesterol. More direct evidence for the formation of coprosterol from coprostanone was obtained by feeding deuterocoprostanone. Coprostanone-4, 5- d_2 containing 3.44 atom % deuterium was prepared by the reduction of cholestenone with deuterium in the presence of active palladium. The deuterium atom at carbon atom (4) is adjacent to the carbonyl group, and consequently the deuterium content of the molecule can be reduced by half by treatment with alkaline alcohol. When coprostanone-4, 5- d_2 was fed to a dog on a meat diet coprosterol containing 0.319 atom % deuterium was found in the stools. In the case of a human coprosterol containing 0.69 atom % deuterium was obtained. Since it is uncertain how much of the deuterium is lost from the molecule, it is impossible to show the exact extent of the conversion. If all the deuterium at carbon atom (4) had been exchanged then 1 atom of deuterium in the recovered coprosterol would correspond to 1 molecule of coprostanone. If no exchange had taken place it would correspond to half a molecule of coprostanone. Thus in the case of the dog the amount of coprosterol derived from coprostanone can vary within the limits of 9.68 and 19.36 %, assuming that no deuterium was lost from carbon atom (5).

The positive finding of deuterium in coprosterol constitutes definite proof of

its origin from deuterocoprostanone. Direct proof of the conversion of cholestenone into coprosterol was also provided by the feeding of deuteriocholestenone. Deutero- Δ^4 -cholestenone was prepared by carrying out a rearrangement of Δ^5 -cholestenone in an alcohol-heavy water containing alkali. Deuteriocholestenone containing 3.1 atoms % deuterium in labile positions was obtained. It was found that deuterium could be introduced into four positions in the molecule by the following mechanism:



It might be expected that the deuterium atoms at (4) and (6) would be stably bound, but actually it was found that all the deuterium was labile in hot alcoholic alkali. This suggests that in alkaline solution there is an equilibrium between Δ^5 - and Δ^4 -cholestenone, with the double bond oscillating between rings A and B. The rate of exchange of deuterium atoms in such positions is slower than that in hydroxyl, carboxyl and amino groups.

In spite of the lability of the deuterium in the deutero- Δ^4 -cholestenone it was found that the compound could still be used. It was fed to a human from whose stools coprosterol was subsequently isolated containing 0.61 atom % deuterium which was not removable by treatment with alcoholic alkali. Here again a positive finding of deuterium in the end-product proves its origin from the deuterium-containing ingested material. The deuterium remained attached to the cholestenone in the organism long enough to be of use, and the reduction of the double bond rendered the deuterium at (4) and (6) stable.

In their paper on the conversion of cholestenone into coprosterol Schoenheimer *et al.* discuss the hypothesis that these ketones might also be concerned in the conversion of cholesterol into bile acids and sex hormones. As in the case of coprosterol, the conversion of cholesterol into bile acids would involve hydrogenation of the double bond to give the *cis*-decalin configuration. The possibility of the conversion of coprostanone into cholic acid was tested (Schoenheimer *et al.* 1936). Coprostanone-4, 5- d_2 was injected up to four times on alternate days into the jugular vein of a dog with a bile fistula, and the bile was collected until 24 hr. after the final injection. It was found to contain an unsaponifiable substance containing deuterium. This they thought was probably unaltered coprostanone. They state that "the presence of the deuterium-containing substance in the fistula bile of our dog is proof that the injected coprostanone (or a substance derived from it) had passed through the liver without being utilized by this organ in the formation of cholic acid". The cholic acid on rigorous purification was found to contain no deuterium. From this result they conclude that coprostanone cannot be converted into cholic acid and cannot therefore be an intermediate in the hypothetical con-

version of cholesterol into cholic acid. They state that while it is possible that the deuterium at carbon atom (4) can be lost by enolization, "no reaction in the hypothetical conversion of coprostanone to cholic acid (shortening of the side chain, reduction of the carbonyl to a hydroxyl group, and introduction of two hydroxyl groups at carbon atoms (7) and (12)) would involve the loss of stable deuterium at carbon atom (5)". They claim that this negative result raises the question of whether the frequently discussed metabolic relationship between cholesterol and bile acid exists, or whether both groups of substances are not synthesized independently in the organism. It is strongly to be doubted, however, whether any importance should be attached to negative results of this nature and it seems inadvisable to draw conclusions from them. The biological interconversion of the steroids, and indeed of all metabolites, is probably far more complex than it would appear on paper, and it is quite possible that during a hypothetical conversion of coprostanone into cholic acid transient intermediate compounds are formed from which the deuterium can be lost before cholic acid is formed. The principle that conclusions cannot be based on negative results when working with deuterium has often been stated by Schoenheimer and his collaborators.

8. *Uptake of deuterium in carbohydrates*

Bonhoeffer and his collaborators have studied the uptake of deuterium into yeast grown on nutrient solutions of various hexoses in heavy water. In their last paper in this series (Günther & Bonhoeffer, 1937) they describe experiments in which yeast was grown aerobically for 4-14 days on nutrient solutions containing 45-60% D_2O and suitable amount of glucose, fructose and mannose. 8-12 mg. dry weight of yeast was sown and 300-350 mg. collected at the end of the experiment. The yeast was then separated into protein, amino acid, glycogen, yeast gum, and cell-wall material and the deuterium content of these fractions determined. The sum of the deuterium contents of the constituents agreed with that of the whole yeast if allowance was made for their quantitative proportion of the total. A discrepancy in this matter which had occurred in earlier work (Salzer & Bonhoeffer, 1936) was found to be due to the fact that in the alkaline hydrolysis of protein the amino acids lost carbon-bound deuterium, thus making it impossible to allow for the deuterium of the protein.

The most interesting results were obtained with the polysaccharides obtained from the yeast. The values given in Table II are the atoms of deuterium % of the stable carbon-bound hydrogen, and they are all calculated on the basis of 50% D_2O in the culture water. The glycogen was obtained from the cell-wall material, the deuterium values for which are given. The values for cell-wall residue could not be determined directly since it was impossible to purify the material; they were calculated from the values for the cell-wall material and for the glycogen on the assumption of the ratio glycogen cell-wall residues = 1:3. These calculated values give the deuterium content of a chemically better defined substance than the glycogen-containing cell walls.

The results in this table show that the deuterium content of the polysaccharides depends on the nature of the nutrient hexose. Glucose, mannose and fructose all serve equally well as nutrient and always give the same yield, quantitatively and qualitatively, of polysaccharides. It seems therefore that interconversions between the different hexoses must take place. Such conversions have been shown *in vitro* by Lobry de Bruyn & van Ekenstein (1895) in the presence of weak alkali. It would appear that under the influence of alkali all three hexoses are in equilibrium with a common enolform. Enzymically the interconversion of glucose and fructose phosphoric esters has been shown by Robison & Tanko (1935).

Table II. *Deuterium content of polysaccharides from yeast*

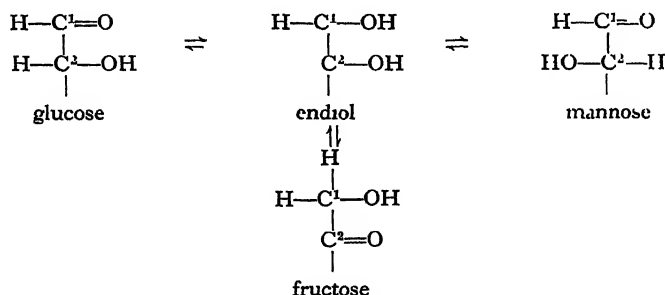
Nutrient	Isolated polysaccharides			Cell-wall residue
	Cell wall	Yeast gum	Glycogen	
Glucose	12.5	11.2	9.1	13.6
Mannose	15.9	5.4	8.6	18.3
Fructose	13.1	11.0	4.3	16.0

The deuterium in the isolated polysaccharide is attached directly to carbon and could thus be taken up only by a chemical process attacking the hexose molecule. It is reasonable to expect, therefore, that the more chemical changes the molecule undergoes, the more deuterium will be built into the polysaccharide. Conversely, the lower the deuterium content of the polysaccharide the more direct was the route from the nutrient hexose. On this principle it will be seen that the shortest route for the synthesis of yeast gum is from mannose, for glycogen from fructose, and for cell-wall residue from glucose. The hydrolysis of yeast gum yields mannose, and the hydrolysis of cell-wall residue yields mainly glucose, but the results with glycogen, consisting as it does of glucose units, are surprising. They are in agreement, however, with the results of Cori (1926), who finds that in the rat liver glycogen is formed more rapidly from fructose than from glucose.

Günther & Bonhoeffer represent glycogen synthesis from fructose and glucose as following in the later stages a common route from a common intermediary which is chemically more closely related to fructose than to glucose. This might be fructose itself, or a functional derivative. The difference in deuterium uptake must take place in the conversion of glucose and fructose to the common intermediary. As regards the conversion of the intermediary into glycogen, they suggest that it might be converted into an active form of glucose not directly derivable from glucose itself, or that it attaches itself, in the form of a fructosephosphoric ester, to a glycogen chain already present and then is converted to glucose.

The deuterium content which results in the polysaccharide even when a favourable hexose is provided is explained by a reversible interconversion of some of the hexose into other hexoses, during which some deuterium must be taken up. If the nutrient hexose is not favourable for the synthesis of a particular poly-

saccharide then all of it must undergo the interconversion. The deuterium uptake during the interconversion process is ascribed to the following equilibrium:



Frequent transition through the endiol would render all the hydrogen attached to carbon atoms (1) and (2) exchangeable. Fredenhagen & Bonhoeffer (1938) have since shown that when the rearrangement of glucose to fructose takes place in alkaline D_2O at room temperature there is no deuterium uptake, but that above 40°C . 1-2 atoms of deuterium are taken up per molecule.

9. Uptake of deuterium in proteins and amino acids

In Copenhagen a study has been made of the uptake of deuterium into proteins and amino acids (Krogh & Ussing, 1936, 1938; Ussing, 1938*b*). When animals are kept with an artificially raised heavy-water content in their body fluids a certain amount of deuterium is taken up into the protein of the various organs. The amount of exchange between the hydrogen of the organs and that of the body fluids can be found by distilling off the water from the organs, and by combustion of the thoroughly dried organs. The percentage of hydrogen atoms of the organs which have exchanged is given by the expression: $\frac{\text{D}_2\text{O in combustion water}}{\text{D}_2\text{O in distillate}} \times 100$. Here it is assumed that the coefficient of distribution of H and D is unity. In proteins approximately 14% of the hydrogen atoms are attached to nitrogen, and are therefore exchangeable with the hydrogen of water (Bonhoeffer, 1934). In a study of the biological uptake of deuterium into proteins it is therefore important to determine how much of the uptake is due to direct exchange and how much is due to vital processes.

Ussing left sterile horse serum to stand with heavy water for 1 hr., 24 hr. and 17 days. After these periods the amount of exchange was found to be 11.8, 13.7 and 16.2% respectively. These figures are in good agreement with the theoretical value for the exchangeable hydrogen of protein. When the protein was denatured by heating at a low pH it was found that the exchange was very slow, being negligible in an hour, and only about 10% of that of soluble protein after 12 hr. The *in vivo* exchange was then studied; a mouse was given an intraperitoneal injection of D_2O and killed 1 hr. later. The amount of exchange in various organs was then determined and found to be: for muscle 4.6%, skin 6.3%, gut 11.4% and liver 13.6%. It will be

seen that the values for gut and liver are close to those for soluble proteins *in vitro*, while the muscle and skin values are much lower. Ussing supposes that the slow exchange of these tissues is due to their solid proteins, such as keratin, elastin, collagen and myosin, which have properties similar to denatured protein. The figures obtained in these experiments were used in an interesting calculation of the myosin content of muscle. The percentage exchange of muscle in 1 hr. is 4.6, that of soluble protein 12; assuming that solid protein, like denatured protein *in vitro*, would exchange no hydrogen in an hour, the percentage of soluble protein in muscle is given by the expression $4.6/12 \times 100 = 39\%$. The percentage of myosin + connective tissue is therefore 61. Noll & Weber (1934) find the myosin to be 40% of muscle, while Smith (1937-8) gives the value of 63% for the proportion of myosin in connective tissue-free muscle. Assuming the amount of connective tissue to be 12%, Ussing's figure on recalculation for connective tissue-free muscle becomes 55%. In Ussing's calculation no account is taken of the partially soluble globulin X of Weber & Meyer (1933). These calculations can only be tentative, since the experiment was carried out with one animal only, and since there is no satisfactory evidence that the muscle and skin had the same access to the injected D_2O within the period of 1 hr. as the other organs.

In 1 hr. experiments with frogs a greater exchange was found for muscle (8.7%) and skin (7.4%) than was found for the mouse, and it was also observed that stimulation and increase in temperature caused an increase in the amount of exchange in muscle.

In experiments of longer duration (1 week or more) a much greater uptake of deuterium in all organs was observed. Part of this deuterium could not be washed out with ordinary water and was therefore attached directly to carbon. This was presumably due to new synthesis of proteins in the heavy-water medium, or to chemical reactions which brought about the labilization of carbon-bound hydrogen. A pregnant rat was injected with heavy water and given heavy water to drink. After 21 days a litter was born; one of the young survived for 21 days, during which time it lived entirely on milk from the mother who was still drinking heavy water. After this time both the young rat and its mother were killed and the amount of exchange determined. It was found that the amount of exchange in the muscle and skin of the young rat was about double the values for the same tissues in the mother.

Krogh & Ussing extended the study of the uptake of deuterium into stable positions in proteins, and in the amino acids obtained by hydrolysis of the proteins. The amino acids isolated from mature rats which had been maintained with a raised level of heavy water in their body fluids were washed repeatedly with water to remove the labile deuterium. None of the remaining stably bound deuterium could be removed by racemization in the presence of barium hydroxide. Since racemization presumably involves a wandering of the α -hydrogen atom it is to be expected that it would exchange under these conditions. Krogh & Ussing conclude therefore that the deuterium built into the amino acids of mature rats is not attached to the asymmetric carbon atom. They claim, on the other hand, to have shown that in young rats the deuterium does probably become attached in this position.

Ussing (1938*b*) fed a casein hydrolysate containing stably bound deuterium to a rat for 3 days. From the deuterium analyses at the end of this period he concludes that at least 2.5% of the muscle protein and 10% of the liver protein was newly formed.

Foster *et al.* (1938) have studied the biological formation of deuterioamino acids. The D₂O level of the body fluids of two groups of mice was raised to 1.5 atoms %. The one group was given a diet of bread and killed after 63 days, and the other a diet of casein and killed after 10 days. Nine amino acids were isolated from the carcasses of the first group and ten from the second. The amino acids isolated were glycine, tyrosine, proline, arginine, glutamic acid, aspartic acid, cystine, leucine, histidine and lysine. They all contained deuterium equivalent to an exchange of 0.5–2.2 atoms per molecule, with the exception of lysine which contained none. With the exception of glycine the deuterium in all the deuterioamino acids could not be removed by treatment with boiling dilute acid. As has been seen in section (2), glycine occupies a special position with regard to the stability of its carbon-bound hydrogen. The fact that the deuterium was built into such stable positions is suggested to be the result of chemical reactions such as synthesis, or deamination and reamination of the corresponding keto acids. The absence of deuterium in lysine is interesting. Foster *et al.* conclude that it proves that no chemical reactions involving the carbon chain of this amino acid had occurred in the animal. This is in agreement with the fact that lysine is known to be an indispensable amino acid. The two other indispensable amino acids which were isolated, histidine and leucine, did, however, contain deuterium. They point out that in the case of histidine it is known that the corresponding α -hydroxy or α -keto acid can be substituted in the diet of growing animals. Enolization of the ketone group in the presence of heavy water, followed by amination will result in "heavy" histidine. The leucine preparation was not separated into its three isomers norleucine, isoleucine and leucine, and it is impossible to state in which of these the deuterium was present. Deuterium could also be introduced into leucine and isoleucine by amination of the corresponding keto acids which can be substituted from them in growth experiments. Foster *et al.* do not claim that the deuterium technique can be used in this way to prove the indispensability of an amino acid, since indispensability does not exclude the possibility of an amino acid being subject to chemical reactions after absorption.

10. Rates of biological synthesis

Schoenheimer & Rittenberg (1936*b*) have carried out experiments in an attempt to determine the rate of synthesis of fatty acids in the organism. Mice whose body fluids were enriched with D₂O were kept on a diet of whole-wheat bread. At intervals groups were killed and the stable deuterium content of their fat depots determined; this was found to increase and to reach a maximum after 6–8 days when the deuterium content of the fatty acids was one-seventh that of the body fluids. After this period the deuterium remained at a constant level and they reasoned that, if uptake of stable deuterium represents a synthesis, the fatty acids were now breaking down as rapidly as they were being synthesized. This break-

down was confirmed by the reverse experiment of feeding mice for 5 days on a diet containing deuterio-fat. At the end of this period some of the mice were killed to serve as controls and the deuterium content of their fat depots determined. The rest of the mice were placed on a fat-free diet and groups were killed after 2, 4 and 6 days. The deuterium content of the depot fat fell at the same rate as it increased in the first experiment, and after 6 days it was one-seventh of the initial value.

It is possible that this apparent synthesis and destruction might be due to repeated hydrogenation and dehydrogenation of fatty acids in the heavy-water medium. The deuterio-fat used in the second experiment was hydrogenated linseed oil, and Schoenheimer & Rittenberg argue that it is improbable that loss of deuterium from this fat could be due to dehydrogenation, since desaturation at the positions where the deuterium had been introduced would lead to the formation of linoleic and linolenic acids, and there is good evidence that the organism is unable to synthesize these acids. That hydrogenation was not responsible for the uptake of deuterium into the fatty acids was shown by the following experiment. The unsaturated deuterio-fatty acids which consist mainly of 9-10 oleic acid were isolated from the depots and split at the double bond into pelargonic acid and the dicarboxylic acid, azelaic acid which is derived from the part of the oleic acid molecule between the carboxyl group and the 9th carbon atom. The azelaic acid contained 0.10 atom % deuterium, which must have been introduced by synthesis of the fatty acids and not by hydrogenation, since the fatty acids of animals are invariably saturated in the portion between the carboxyl group and the 9th carbon atom.

The "half-lifetime" of the fatty acid molecule, that is, the time in which the deuterium content reaches half its maximum value, was obtained from a curve constructed from the data on the deuterium uptake; it was found to be about 3 days.

When the experiments were repeated and extended Rittenberg & Schoenheimer (1937*b*) found that the deuterium level of the fatty acids now became approximately constant after 30-40 days, and that the half-lifetime was from 5 to 9 days. These widely different results support the view that rates of biological synthesis of fatty acids, likely to be influenced by a number of factors, are extremely variable.

Similar experiments were carried out with cholesterol, in which the maximum deuterium content was reached after 60 days. This maximum value was 0.78 atom %, while the deuterium content of the body fluids was 1.7 atoms %. The concentration of deuterium in the cholesterol is therefore about half that in the body fluids, from which Rittenberg & Schoenheimer conclude that in the various stages of cholesterol synthesis half its now stable hydrogen atoms were exchangeable. They infer that chemical reactions must have happened at a great number of carbon atoms, and that the cholesterol was therefore probably formed by the coupling of a number of smaller molecules.

11. *Limitations of the deuterium technique*

The deuterium technique suffers from limitations which are peculiar to this particular isotope. Although it is often possible to predict the stability of the carbon-hydrogen bond in molecules of known constitution, or to test this stability

by experiments *in vitro*, the possibilities for exchange under biological conditions are not known and cannot always be predicted. It is not suggested that the chances for direct physical exchange of carbon-bound deuterium are different *in vivo* and *in vitro*, and the possibility of a hydrogen-labilizing enzyme probably is remote; but the probability of chemical reactions taking place *in vivo* which might ultimately place the deuterium in labile oxygen or nitrogen-bound position is unfortunately always present.

Transient intermediates, not detectable by the means at present at our disposal, but in which the deuterium for one reason or another is labile, may be formed. Most metabolites that have been studied are of such a degree of complexity that the number and size of regions in the molecule in which the hydrogen is not reactive, or cannot be brought into reactive positions by chemical reactions, is small. This restricts the useful application of deuterium to a limited number of biologically interesting compounds, such as the relatively inert fatty acids and steroids. With the steroids one is faced with the hitherto incompletely solved difficulty of introducing the isotope into stable positions.

A review of the work which has been done bears out the fact that the value of the experiments so far carried out would seem to lie not so much in the fact that new discoveries have been made, but that results previously obtained by other techniques have been confirmed, and sometimes placed on a firmer basis.

III. HEAVY NITROGEN

The heavy isotope of nitrogen with atomic weight 15 (N^{15}) has now been concentrated from a natural abundance of 0.368% up to 15% (Thode *et al.* 1938). The biological application of this isotope was first reported in 1937 (Schoenheimer *et al.*), and its use as an indicator in the study of amino acids bears great promise. Since it differs even less than deuterium from its natural analogue it is highly improbable that it is distinguishable by the organism, though Schoenheimer & Rittenberg (1939) have found that the N^{15} content of various natural amino acids differs very slightly from that of air.

The determination of heavy nitrogen is more complicated than that of deuterium since a mass spectrometer is required (Rittenberg *et al.* 1939). The nitrogen of the sample is converted into ammonia by Kjeldahlization, and the ammonia is then converted into molecular nitrogen by the action of alkaline hypobromite under reduced pressure. The nitrogen is collected in a sealed tube, and at a suitable time it is admitted into the vacuum tube of the mass spectrometer, where it is ionized by a stream of electrons from a heated filament. The singly charged $N^{14}N^{14}$ and $N^{14}N^{15}$ ions ("doublets") are then separated into a mass spectrum in a magnetic field under the influence of a known and variable negative potential. By varying this potential doublets of the desired mass can be made to enter a slit at will and strike an insulated collector plate. The intensity of the ion current which passes from this plate is a measure of the number of ions striking it, and therefore of the proportion of a doublet of particular mass in the sample. By this means it is

possible to determine a concentration of N^{15} 0.003 % in excess of normal in a 1 mg. sample of nitrogen.

When N^{15} is used as an indicator in the amino group of an amino acid it is essential that the nitrogen of the amino group should not exchange with the nitrogen of other nitrogenous compounds. Such a possibility is in any case more restricted than in the case of hydrogen, since the number and amount of nitrogenous compounds is far less than the number of compounds containing hydrogen. Keston *et al.* (1939) have heated together for 1-104 hr. at temperatures from 37 to 105° C. the following pairs of compounds: (1) amino acid—ammonium salt, (2) amino acid—amino acid, (3) hippuric acid—amino acid, and (4) urea—amino acid. In each case the nitrogen of one of the components was enriched with respect to the heavy isotope; no exchange was observed. It seems that the isotope can only enter the molecule by chemical reactions, under biological conditions by such processes as deamination and reamination or by "umaminierung" (see Braunstein & Kritzmann, 1937).

Since the concentration of N^{15} is a costly process the preparation of amino acids containing this isotope must be carried out in a way which permits the maximum recovery of nitrogen. Schoenheimer & Ratner (1939) have prepared a series of *dl*-amino acids containing 1.9 atoms % N^{15} in excess of normal abundance by the methods of Knoop & Oesterlin (1927) and Gabriel & Kroseberg (1889). The isotope is supplied in the form of an ammonium salt, and in each case a quantitative recovery of the nitrogen in the form of amino acid and unused ammonia is obtained, with a high yield of amino acid on the basis of ammonia used.

Foster *et al.* (1939) have showed that dietary inorganic ammonia appears at least to a slight extent in the amino acids of body proteins under conditions of protein starvation. Two rats were kept on a low protein diet containing 3 % yeast protein and 2.3 % nitrogen as ammonium citrate (1.21 atoms % N^{15} in excess of normal) for 5 days. During this period the rats lost weight; these unphysiological conditions were chosen since it was thought that the rats in need of protein might use inorganic ammonia for amino-acid synthesis. Creatine (0.036) and the following amino acids were isolated from the carcasses: glycine (0.050), glutamic acid (0.085), aspartic acid (0.067), proline (0.037), histidine (0.012), arginine (0.033) and lysine (0.003). The figures in brackets represent the atoms % N^{15} in excess of normal. The figure for lysine is negligible, while the figures for glutamic acids are high; these results are similar to those obtained with deuterium and they are significant in view of the known indispensability of lysine and in view of the fact that the dicarboxylic acids are said to be intermediates in the formation of other amino acids (Braunstein & Kritzmann, 1937). The arginine was hydrolysed with strong alkali to ammonia and ornithine and all the isotope was found in the ammonia, and therefore must have been in the guanido part of the arginine molecule.

The value of 0.114 % for the amide nitrogen which was liberated as ammonia during the protein hydrolysis shows that more than 10 % of the amide nitrogen was derived from the ingested inorganic ammonia.

The histidine was converted by treatment with nitrous acid into imidazole lactic

acid which was then found to be free of the heavy isotope (Schoenheimer *et al.* 1939). The imidazole ring was therefore probably not newly formed and the nitrogen of the inorganic ammonia must have entered the histidine molecule by a process of deamination and reamination at the α -carbon atom.

Two amino acids which were enriched with respect to N^{15} were fed, namely, tyrosine (Schoenheimer *et al.* 1939) and glycine (Rittenberg & Schoenheimer, 1939). Synthetic *dl*-tyrosine containing 2.04 atoms % N^{15} in excess of normal was fed with casein in a stock diet to a rat for 6 days. 50–60% of the isotope was recovered in the urine, while the rest was found in the tissues, almost all in the form of protein nitrogen. The liver protein contained three times as high a concentration of the isotope as the rest of the carcass. The protein was hydrolysed and the distribution of N^{15} in some of the constituents determined. 20–25% of the total isotope of the proteins was found in the tyrosine.

The isotope was also found in a mixture of glutamic and aspartic acids, in histidine, arginine and in the amide nitrogen, but again not in the lysine. It was not yet quantitatively accounted for, but other amino acids were not isolated. In this experiment the arginine was isolated from the liver and it was found that the guanido part of the molecule contained nearly as much N^{15} as the urinary urea. This is in excellent agreement with the urea cycle of Krebs & Henseleit (1932).

The experiment with "heavy" glycine was carried out in order to determine whether dietary glycine is used directly in the detoxification of benzoic acid or whether tissue glycine is preferred. It is known that when benzoic acid is fed hippuric acid is excreted even when glycine is not included in the diet. Adult rats were given benzoic acid together with two equivalents of isotopic glycine; the urinary hippuric acid was found to contain N^{15} , but only a third as much as the dietary glycine. It was concluded therefore that two-thirds of the glycine which was used in the detoxification of benzoic acid must have been derived from another source, but the experiment does not show whether this was taken from the proteins or especially synthesized.

IV. OTHER ISOTOPES

The heavy isotope of oxygen has recently been applied to a biological problem. It can also be estimated in water by a density determination, but this method is not as sensitive as it is in the case of deuterium. Some exchange reactions of oxygen have been studied; thus Datta *et al.* (1937) have found that the $-\text{OH}$ ion is the active agent in the exchange of oxygen between the sulphate ion and water, which is slow at neutral *pH*. Cohn & Urey (1938) find that the oxygen of $-\text{OH}$ and $-\text{COOH}$ groups does not exchange while the oxygen of the carbonyl group of acetone does. On the other hand, Mears (1938) finds a slow exchange of two oxygen atoms in glycine hydrochloride, but no exchange with glycine. Titani *et al.* (1938) find no exchange between the carbonate ion and water.

Day & Sheel (1938) have used heavy oxygen in an experiment to see how far oxygen of respiratory CO_2 is identical with oxygen inspired as molecular oxygen. Rats were kept in an artificial atmosphere containing heavy oxygen equivalent to

water of 300 parts per million excess density and the expired CO_2 was found to contain oxygen equivalent to water of 40 parts per million excess density. Two possible courses are open to the respiratory oxygen: (a) it can enter directly into the oxidation of the carbon of metabolites and be expired as CO_2 , or (b) it can combine with the hydrogen of water, while the originally combined oxygen of the water can carry out the direct oxidation of the metabolites. Since the latter mechanism would lead to a complete absence of the isotope in the expired CO_2 , it is concluded that the former mechanism must operate. The dilution of the isotope in the expired CO_2 can be due either to a functioning of the second mechanism, or to an oxygen exchange between oxygen and water, or to both.

The concentration of the heavy isotope of carbon has been effected, and its biological application will probably be announced in the future. This is obviously the ideal isotope to use in biochemical work, since the study of intermediary metabolism is essentially a study of the fate of the carbon skeleton of metabolites. Here the possibility of exchange will present no problem, but the chief obstacle will be the synthesis of biologically interesting compounds from CO_2 , the form in which the isotope is concentrated.

V. SUMMARY

1. The application of stable isotopes as indicators does not differ in principle from the application of radioactive isotopes, though the determination of the latter is generally more convenient.

2. Since deuterium has several physical properties which are markedly different from those of the more abundant isotope of hydrogen it has definite biological effects in high concentrations. In low concentrations there are no appreciable biological effects, and it is not expected therefore that the application of deuterium will be limited in this respect.

3. Deuterium is used as an indicator in two ways: (a) it is used as a label directly attached to the carbon atoms of metabolites, (b) reactions are carried out in a heavy-water medium and the uptake of deuterium in the end-products is determined.

4. An account is given of the methods for the determination of deuterium and of some of the methods for preparing deuterio-organic compounds. The deuterium content of an organic compound is generally calculated from the density of its combustion water.

5. The stability of the carbon-hydrogen bond is discussed; although it is often possible to predict the stability of this bond *in vitro*, it is concluded that the possible wandering of the label under biological conditions would limit its useful application to a few types of compounds, such as the relatively inert fatty acids and steroids.

6. The retention time of a molecule of water in the human organism has been found to be 14 days by experiments which involved the ingestion of heavy water and the determination of the deuterium content of the urine. The permeability of frog skin to water was found to be 400 days at 0°C .

7. The metabolism of trideuteroacetic acid by yeast was studied. From the deuterium content of succinic acid isolated it was concluded that the acetic acid

was converted into succinic acid, but the deuterium values gave no clue as to the mechanism of this conversion. The steroids isolated from the yeast had a higher deuterium content than the fatty acids, and it was concluded therefore that they were not derived from the fatty acids, but probably from the acetic acid.

8. In feeding experiments with deuterio-fatty acids it was found that they were deposited before being burned, even when the fat content of the diet was abnormally low. Caproic and butyric acids were disposed of by mice within 8 hr., and this disposal did not consist of a conversion into higher fatty acids. From the deuterium content of lipoids and glycerides isolated after the feeding of deuterio-fat it was concluded that ingested fatty acids play an active part in the metabolism of these compounds. The source of increased liver fat which results from starvation, exposure to carbon tetrachloride vapours, and injection of an extract of the anterior pituitary gland, was traced to the depot fat, and it was also concluded that this is not the source of increased liver fat resulting from a high carbohydrate diet. Desaturation and saturation of fatty acids were proved to take place by following the deuterium content of the unsaturated fatty acids of mice which had been fed saturated deuterio-fatty acids, and vice versa. It was shown that stearic acid is converted by the organism into palmitic acid.

9. It was shown that cholestenone and coprostanone are converted by the dog and by man into coprosterol. These two substances can be intermediates in the conversion of cholesterol into coprosterol *in vitro*.

10. When yeast was grown in a heavy-water medium on various nutrient hexoses it was found that the least deuterium was incorporated in the glycogen subsequently isolated when the nutrient hexose was fructose. It was accordingly concluded that the most direct conversion of a hexose to glycogen was from fructose, and not from glucose. The deuterium values did not give a satisfactory clue to the mechanism of this conversion.

11. From the deuterium content of the muscle of a mouse injected with heavy water the myosin content was calculated to be 55 %. In experiments of long duration a considerable amount of deuterium was built into the protein of mice, but no conclusions were drawn from the results. A series of deuterioamino acids were isolated from mice which had been maintained with an artificially raised level of heavy water in their body fluids. No deuterium was found in the indispensable amino acid, lysine.

12. Attempts were made to determine the rates of biological synthesis and breakdown of fatty acids and cholesterol from the deuterium content of these compounds isolated from mice which had been kept for various periods with heavy water. The results thus obtained were not conclusive.

13. Heavy nitrogen has also been used as an indicator. It was found that the nitrogen of ingested ammonium salts and of ingested *dl*-tyrosine was incorporated in a large number of amino acids, with the exception of lysine. In some cases this incorporation was shown to take place by a process of deamination and reamination. It was shown that the glycine of urinary hippuric acid is not all derived from ingested glycine, even when a superfluous amount is fed.

14. The heavy isotope of oxygen has been used in a study of the source of the oxygen of respiratory carbon dioxide.

15. The possibilities of the application of the heavy isotope of carbon are mentioned.

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